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A quantitative study on blood vessels and other smooth muscle effectors in the cat

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CHAPTER I

General introduction

The autonomic control of various structures which is effected by the sympathetic nervous system is unique in the sense that it has a neurogenic component in the thoracolumbar sympathetic outflow and, at the same time, a blood-borne understudy in the adrenal medullary hormones. Since the theory of a humoral transmission of nerve impulses at the sympathetic neuroeffector junctions was established (ELLIOT 1904, LOEWI 1921 and CANNON and URIDIL 1921), these two efferent links of the sympathetic nervous system have often been brought together under the common heading of the 'sympathico-adrenal system'. A third possibility by which any effector might be influenced by the sympathetic nervous system has been claimed, especially by CANNON and his group (for references see the monographs by CANNON and ROSENBLUETH 1937, 1949 and by ROSENBLUETH 1950), since it was demonstrated that the ergones released at sympathetic postganglionic nerve endings, at least under certain circumstances, might diffuse into the blood stream and induce distant effects similar in nature to those elicited by the discharge from the adrenal medulla.

Thus we have three possible pathways in the motor control of effector units that are subordinate to the sympathetic nervous system. It has been commonly understood that there is a more or less generalized co-operation between these three main routes of sympathetic ergone supply to the effectors. This concept is evident e. g. from the common denotion of the adrenal medullae as the 'loud pedal' of the sympathetic nervous system, reinforcing the effects of the direct sympathetic innervation. This statement might picture the actual situation but it is based on a rather meager knowledge about the relative potency and the quantitative relationships between the abovementioned components. The major

aim of the present investigation is to make a quantitative study of the full range of control that each of these three different pathways of sympathetic control will exert on various effectors. The author has been primarily interested in the sympathetic control of the circulatory system but additional observations were made on other smooth muscle effectors such as the nictitating membrane and the dilator muscle of the iris.

The adrenal medulla is a part of the sympathetic nervous system. From an embryological point of view the secretory cells of the adrenal medulla are comparable to postganglionic neurons of the thoracolumbar sympathetic outflow. In addition, the secretory activities of these medullary cells are primarily governed by praeganglionic sympathetic neurons running mainly in the splanchnic nerves. Thus, the two main competitors in the sympathetic motor control of various effectors — the direct innervation and the adrenal medullae — must have a limiting factor in common, namely the capacity of sympathetic nerve fibres to transmit nerve impulses. The upper limit of the rate of impulse discharge in the sympathetic nervous system has been studied with different methods by several investigators. The most direct information has been obtained by recording action potentials from individual sympathetic motor nerve cells. From such studies BRONK, FERGUSON, MARGARIA and SOLANDT (1936) concluded that the normal discharge rate of the sympathetic motor nerve cells was remarkably low and seldom higher than 10 to 20 impulses per second. It has also been repeatedly demonstrated that a ganglion cell in a sympathetic ganglion does not respond repetitively to a single praeganglionic volley, and in accordance with this it was stated by BRONK, TOWER, SOLANDT and LARRABEE (1938) that there will be a strict one-to-one relationship between the rate of stimulation of praeganglionic nerve fibres and the impulse discharge recorded from postganglionic neurons. In experiments where praeganglionic fibres were stimulated at rates higher than about 20 impulses per second the postganglionic action potentials became progressively smaller (for refs. see BRONK 1939). PERRY (1953) in a study of the amount of acetylcholine released in an eserinized sympathetic ganglion upon praeganglionic stimulation reported that the rate of deterioration in output per volley was much more rapid for higher frequencies of

praeganglionic stimulation. These phenomenons might be related to an exhaustion of the cholinergic transmission of nerve impulses across the ganglion synapses when the praeganglionic fibres were stimulated at supraphysiological rates. Another limiting factor at the synaptic level regarding the upper limit of impulse discharge in the sympathetic nervous system is the duration of the refractory period of the sympathetic ganglion synapse. This was stated to average 20 to 30 msec (BISHOP and HEINBECKER 1930, 1932), and consequently the ability of the ganglion synapse to transmit nerve impulses must have a theoretical upper limit at about 30 to 50 impulses per second. The figures on impulse frequency in the sympathetic nervous system given by BRONK and co-workers are probably not supposed to be accepted as altogether accurate. That is because of the hazards in recording action potentials from individual sympathetic motor nerve cells. The tiny and fragile sympathetic fibres make this isolation a difficult task.

Another more indirect approach to the same problem of the 'physiological' discharge rate of the sympathetic nervous system which has been repeatedly tried (for refs. see e. g. ROSENBLUETH 1932, the review by BACQ 1935 and the monographs by CANNON and ROSENBLUETH 1937 and ROSENBLUETH 1950) is to determine which frequency of sympathetic nerve stimulation that gives a maximum response of the corresponding effector. In mammals, this effective upper limit of sympathetic nerve fibre discharge generally was found to be around 10 to 25 impulses per second, and in cold-blooded animals it was still lower (BOZLER 1936). As there are no sane reasons why excitation at supramaximal rates ever should occur in the intact organism these experiments give an indirect clue as to the normal discharge rate of sympathetic nerve fibres. The figures reported might, however, still be too high. Any failure to stimulate *all* nerve fibres innervating an effector will reduce the response and consequently the frequency-response curve for this effector must be flattened and shifted to the right. This is obvious when it is considered that postganglionic axon ramifications from several neurons might influence a single effector cell (ECCLES and MAGLADERY 1937 a, b). Experimental evidence for such a convergence principle concerning the peripheral autonomic innervation was also presented by HILLARP (1946). Such a failure

to excite all neurons converging upon a given effector must be a rather inevitable experimental error in many studies along this line and may falsely give the impression that comparatively high frequencies are necessary to obtain a maximum response of certain effectors.

Still another approach to the same problem was practised by FOLKOW (1951, 1952). In a sense, his method might be claimed to apply the same principles as those involved in any type of quantitative biological assay. Following an intense reflex activation of the central structures in charge of the sympathetic vasoconstrictor nerve outflow there was a prominent peripheral vasoconstriction. By continuously recording the blood flow of an isolated skeletal muscle region this vasoconstriction could be quantitatively evaluated as a change in peripheral resistance of this vascular area and expressed in terms of relative units for peripheral resistance. The extent of this vasoconstriction must be graded to the rate of impulse discharge in the corresponding sympathetic vasoconstrictor nerve fibres. The answer to the question of what rate of impulse discharge that was responsible for the vasoconstriction recorded could be given by reproducing a similar vasoconstriction by electrical stimulation of the corresponding vasoconstrictor nerves at known frequencies. The maximal rate of impulse discharge in the vasoconstrictor nerve fibres following intense reflex activation of the vasomotor centre is probably of about the same order of magnitude as the frequency found necessary to reproduce the response of the vascular area. It was concluded by FOLKOW that the normal peripheral resistance of the small vessels is maintained by a quite low rate of impulse discharge, probably around 1 to 2 impulses per second. The increase of impulse discharge accompanying a strong reflex excitation of the vasomotor centre could be calculated to approximate 3 to 5 impulses per second, and no evidence was ever obtained in support of an increased rate of impulse discharge exceeding 6 to 8 impulses per second.

Thus the combined evidence obtained by a number of investigators using quite different methods strongly supports the view that under basal conditions the tonic activities of the sympathetic nervous system are maintained by a quite low discharge rate of nerve impulses in the sympathetic nerve fibres. There are further good reasons

to assume that the normal range of impulse discharge is rather limited, most probably never exceeding 10 impulses per second during any type of reflex excitation of the sympathetic nervous system. This is a rather slow rate of impulse traffic when compared with what is known to occur in the larger motor nerve fibres innervating skeletal muscle cells, and it is probably related to the delicate structure and small diameter of the nerve fibres constituting the peripheral sympathetic innervation. Still it is perfectly possible to provoke almost maximal responses of most effectors by electrical stimulation of the corresponding sympathetic nerve fibres up to that rate. On the basis of the experimental evidence that has been referred to, I have accepted as a working hypothesis that the upper limit of impulse discharge in the sympathetic thoracolumbar outflow is something around 10 impulses per second.

To carry through a comparative, quantitative investigation of the full range of motor control which the two major efferent components of the sympathico-adrenal system — that is the corresponding sympathetic innervation and the adrenal medullae — will exert on different effector systems the prime requisite must be a common reference. In the present study both components have been activated at different frequencies within the physiological discharge range of the sympathetic nervous system and the rate of electrical stimulation applied has been adopted as the standard of comparison. By these procedures it will be possible, in the first place, to get some information about the capacity of each of the two components regarding the motor control of different effectors. At the same time, their relative potency might be evaluated by a comparison of the responses obtained when each of the two components were stimulated separately at similar rates.

The fact that the adrenal medulla from an embryological and functional point of view makes up an integrative part of the sympathetic nervous system does not, of course, necessarily imply that the neurogenic and blood-borne components are closely linked up and active to about the same extent in every activation pattern of the sympathetic nervous system. But in a multitude of stressful conditions the sympathico-adrenal system is brought into action as a whole. This is the fundamental characteristic of the 'emergency reactions' (see e. g. CANNON 1929). The structural basis for this diffuse

and widespread mode of action is the marked divergence principle which applies to the thoracolumbar sympathetic outflow. So for instance BILLINGSLEY and RANSON (1918) calculated that in the superior cervical ganglion of the cat each praeganglionic nerve fibre made contact with 32 postganglionic neurons. A similar divergence principle appears to exist at the peripheral neuro-effector junctions as well. According to HILLARP (1946) each postganglionic neuron innervates a great number of effector cells. In addition, the blood-borne catechols from the adrenals will be quite diffusely distributed.

Quantitative estimates of the full range of the direct sympathetic motor control of various effectors can be found in studies where the responses of the effectors were recorded at different rates of sympathetic stimulation. Mostly, however, such studies deal with effectors such as the nictitating membrane (see e. g. ROSENBLUETH 1950) whereas the sympathetic control of the regulating smooth muscle cells in the blood vessels has been somewhat ignored. Technical difficulties may partly account for this fact. Most of the studies on the range of sympathetic control of different vascular areas are of quite recent origin. A study concerning the sympathetic constrictor and dilator innervation to the blood vessels of a skeletal muscle region in the cat was published by FOLKOW (1951, 1952). GIRLING (1952) recorded the responses of the cutaneous vessels of the rabbit's ear following stimulation of the cervical sympathetic trunk. CELANDER and FOLKOW (1953) made a comparison of the sympathetic constrictor nerve fibre control of cutaneous and muscular blood vessels in the cat. In short, these studies might be claimed to demonstrate that drastic increases of regional peripheral resistance could be obtained by a surprisingly slow rate of sympathetic nerve stimulation. Thus, in some animals it was possible to bring about a reduction of cutaneous blood flow almost to standstill by a rate of sympathetic stimulation that did not exceed 10 impulses per second (CELANDER and FOLKOW 1953).

Regarding the adrenal medullae we have rather much of experimental evidence, also from older papers, concerning the amounts of hormones that are released to the blood stream during various experimental conditions (see e. g. MCDOWALL 1938, DUNÉR 1953 and EULER and FOLKOW 1953). However, since it was established that noradrenaline might constitute an important fraction of the total catechol output from the adrenals most earlier quantitations

of the adrenal medullary secretion in terms of adrenaline loose their accuracy. This is because adrenaline and noradrenaline have subsequently been demonstrated to show more or less pronounced quantitative differences in their actions on different test preparations (see e. g. EULER 1950). The demonstration of noradrenaline as an active principle of the adrenal medullary secretion originates from a suggestion by HOLTZ, CREDNER and KRONEBERG (1947) and has since been repeatedly confirmed (see e. g. EULER 1951). The functional significance of the occurrence of noradrenaline in the adrenal medulla is by no means settled. The fact that during foetal life the mammalian adrenal gland shows a preponderance of noradrenaline (HÖKFELT 1951/52), the demonstration of large amounts of noradrenaline in the poorly differentiated cells of phaechromocytomata (HOLTON 1949 and others) and the capacity of the adrenal gland to methylate noradrenaline into adrenaline (BÜLBRING 1949, BÜLBRING and BURN 1949) support the view that noradrenaline might be an intermediary step in the synthesis of adrenaline. However, inasmuch as noradrenaline has been demonstrated to be liberated into the blood upon reflex excitation of the adrenal medulla the classification of noradrenaline as a true hormone is well justified. During recent years there has been a growing tendency to differentiate the activities of the adrenal medulla in two main categories — that is the production of adrenaline versus the production of noradrenaline. In his important paper HILLARP (1946) reported that, after the adrenal medulla had been excited by convulsive doses of insulin, apparently quite inactive cell complexes could be seen in the middle of aggregates of cells that must have been strongly activated. The explanation offered by HILLARP was that the hypoglycaemia induced by insulin did not excite all the neurons which constitute the center for adrenal medullary gland cells. Recently HILLARP and HÖKFELT (1953) obtained direct cytological evidence that adrenaline and noradrenaline exist in different cell complexes of the adrenal medulla. The finding of HÖKFELT (1953) that insulin hypoglycaemia brings about a selective increase in the medullary output of adrenaline is in good agreement with the previous observations by HILLARP on the selective stimulation of some cell complexes only. This functional differentiation of the cells of the adrenal medulla appears to have a correlate in the neurons

constituting the central structures that govern the activities of the adrenal medulla. It has been demonstrated that the total amount and the relative percentage of adrenaline and noradrenaline in the medullary secretion might differ according to which mode of reflex activation that was applied (see e. g. EULER and FOLKOW 1953). Analysis of the catechol output in the adrenal venous outflow following upon localised electrical stimulation in the hypothalamic region (FOLKOW and EULER 1954) gave some evidence to show that the central control of the adrenaline- and noradrenaline-producing fractions of the adrenal medulla have a separate representation in different hypothalamic areas.

These investigations make up a rather firm basis for the assumption that adrenaline and noradrenaline are two separate hormones, produced by separate cells in the adrenal medulla, and liberated upon different types of reflex activation by the interaction of separate nervous structures at various central levels.

Among the various adverse conditions which are known to provoke a speeding up of the adrenal medullary secretion, those which are met with in states of circulatory failure are of a special relevance when it comes to an evaluation of the importance of the adrenal medullae in the circulatory homeostasis. BEDFORD (1917) claimed that 'increased quantities of epinephrin are thrown into the blood during conditions of low blood pressure and shock'. This observation has since been confirmed by a number of investigators (for refs. see e. g. MCDOWALL 1938). Later on, it became evident that this reflex excitation of the adrenal medullae, at least partly, could be attributed to an inhibition of the tonic baroreceptor activities which parallels the lowering of the blood pressure (HEYMANS 1929). HOLTZ and SCHÜMANN (1948, 1949) put forward the view that a decreased baroreceptor discharge brought about a selective release of noradrenaline from the adrenals. This observation indicates that noradrenaline from the adrenals might be a factor of major importance in circulatory homeostasis (see e. g. the paper by HOLTZ and SCHÜMANN 1949). This claim of a selective excitation of the noradrenaline producing units of the adrenal medulla has not been supported by later experiments. So for instance BRAUNER, BRÜCKE KAINDL and NEUMAYR (1950) reported an increase of catechol secretion of the adrenals from 0.082 to 0.213 μg/kg/min during

clamping of both carotides, but there was no evidence of any major change in the relative proportions of the two catechols. Their results are in accordance with those published by KAINDL and EULER (1951) and EULER and FOLKOW (1953). Furthermore, the latter authors found it necessary to make the inhibition of baroreceptor regions complete by sectioning of the vagal nerves. Otherwise no significant increase in medullary output occurred whatsoever. The problem was re-investigated by HOLTZ and co-workers (1952 a) by a more direct method and they agreed that the relative percentage of noradrenaline in the medullary venous outflow did not change following clamping of the carotid arteries when compared with the 'resting' secretion. These observations do not support the assumption that noradrenaline-producing cells of the adrenal medulla should be selectively engaged in the circulatory homeostasis. The increase of catechol release during occlusion of the carotid arteries seems to be rather moderate and goes for adrenaline as well as noradrenaline.

In many states of a heavy reflex excitation of the adrenal medullae, e. g. such as occurs during fulminating asphyxia, there is a pronounced increase of the catechol output, but commonly without any change of the relative proportions between adrenaline and noradrenaline (RAPELA and HOUSSAY 1952, EULER and FOLKOW 1953), indicating that both types of medullary cell complexes are brought into action. The catechol secretion during asphyxia accounts for the highest figures on catechol output following upon any type of reflex activation of the adrenal medullae and the total amount of catechols released during asphyxia may exceed 2 μg/kg/min. These figures might give some information about the upper limit of catechol secretion during 'physiological' conditions but still it must be admitted that it is difficult to judge the extent of adrenal medullary excitation from experiments of this type. In order to get some information about what might be called the theoretical upper limit of catechol secretion from the adrenals a series of experiments were included in the present study where the sympathetic nerve fibres to the adrenals were stimulated at various frequencies and the amounts of catechols released were quantitatively assayed. In addition, the amount released by each single shock stimulus was calculated. A similar but too fragmentary study was published by ROSENBLUETH (1932).

Many authors have tried to get an idea about the quantitative effects of the adrenal medullary secretion by eliciting different kinds of reflexes before and after extirpation of the glands. In case of the circulatory system mostly only the blood pressure has been used as an indicator (see e. g. HEYMANS and BOUCKAERT 1934, and GLEY and QUINQUAUD 1917/18, 1921). Changes in blood pressure, however, do not picture properly the vascular changes that might be induced by the vasomotor nerves or the medullary hormones. The fact that the blood-pressure level is a poor indicator as to what might be going on within the circulatory system is well illustrated by some of the figures in the papers by ELIASSON, FOLKOW, LINDGREN and UVNÄS (1951) and FOLKOW and GERNANDT (1952). So for instance, hypothalamic stimulation might evoke negligiable blood-pressure changes, behind which one finds most dramatic redistributions of blood flow. Thus, a marked vasoconstriction in some areas might well, from a haemodynamic point of view, be more or less completely balanced by vasodilatations in other vascular regions. Furthermore, at intense increases of the total peripheral resistance due to extensive vasoconstrictions in different regions it cannot be expected that the blood-pressure rise will run parallel with the constrictor effects as the heart muscle most probably will not be able to maintain an unchanged cardiac output at these heavy loads. Thus, it might be perfectly possible that — for instance during a fivefold increase of peripheral resistance — the heart will only be capable of raising the blood pressure to about double the initial level. More intricate, analyzing methods are therefore necessary in order to obtain closer information. Cross-circulation experiments in which the adrenal venous outflow of the donor is directed to the blood stream of another animal is an excellent method to study the effects of the adrenal medullary secretion (for refs. see e. g. MCDOWALL 1938). So for instance asphyxia of the donor brought about an increase of adrenal medullary output which in the recipient animal was manifested by hyperglycaemia, inhibition of intestinal peristalsis and a decrease of kidney volume and spleen size (HOUSSAY and MOLINELLI 1926). However, even from experiments of this type it is almost impossible to get an idea about the relative importance of the adrenal medullae in the sympathetic control of various effectors because there is no standard of comparison regarding the effects

of the corresponding sympathetic innervation on the same tissues. In fact, there is an almost complete lack of knowledge as to the quantitative roles of the two main contenders — the direct sympathetic innervation and the adrenal glands — when compared with each others on clearly defined substrates during similar experimental conditions in one and the same animal and applying methods which permit an accurate quantitative evaluation of the responses. Some indirect guidance might be obtained from available experimental data, but not very much, and few investigators appear to have realized the importance of stimulating the sympathetic innervation of an effector and the adrenal glands at similar rates. This must be the first thing to do to allow a rough general estimate. To make such a quantitative comparative study more complete it is necessary to add an analysis of their relative effects in a true physiological reflex excitation of the sympathetic nervous system.

Regarding the third possible influence on the effectors — that is the mechanism of an 'overflow' into the circulating blood of the adrenergic transmittor substances released in some areas by which remote effects on other areas might be induced — the available literature provides still more confusing and contradictory evidence. Thus, in some papers it has been claimed that the overflow of the transmittor substances is a factor of considerable importance for ensuring a reinforcement and generalization of sympathetic effects (see e. g. the review on this matter given by ROSENBLUETH in his monograph 1950). In other papers ROSENBLUETH argues that the importance of the abovementioned mechanism during normal conditions must be negligible (see e. g. LIU and ROSENBLUETH 1935). However, the effects of this overflow-mechanism have been studied on various substrates that have been sympathetically decentralized after the adrenals have been ruled out. Furthermore, these substrates have — with few exceptions — been rendered abnormally sensitive to sympathomimetic compounds by some sensitizing procedure such as previous denervation or the administration of cocain. It is obvious that no safe information about the quantitative importance of the overflow-mechanism in the normal organism will be obtained from experiments of this type. There is still the necessity of a standard of comparison and the relative roles of the three separate ways of sympathetic ergone supply can only be judged from a direct

comparison of the extent of the responses that each of them will bring about on different substrates during otherwise identical experimental conditions.

As will be evident from this short survey, quantitative aspects of the sympathetic motor control of various smooth muscle effectors are mostly of a quite recent origin and partly still rather fragmentary. The present study is an attempt to extend this knowledge by applying methods which allow a reliable quantitative recording of the responses of various smooth muscle groups. In the first place, this could be achieved by a comparison of the responses of various effectors obtained by electrical stimulation of the corresponding sympathetic innervation and the secretory nerves to the adrenals separately at similar frequencies within what might be considered the physiological discharge range of the sympathetic nervous system. Thus the capacity of the two main efferent links of the sympathetic nervous system in this motor control as well as their relative efficiency and probable physiological importance could be evaluated. In addition, a series of experiments was included in this study where a true reflex excitation of the sympathetic nervous system was induced. This allowed a quantitative estimate of the share of the direct sympathetic innervation and the adrenal glands in the motor adjustments of various effector groups that characterized the sympathetic response. Information about the integrative function of the sympathetic nervous system with some quantitative aspects might thus be obtained. A preliminary report on this study was published in *Nature* (CELANDER 1953).

CHAPTER II

The magnitude of the adrenal medullary discharge

In contrast to most other endocrine tissues the adrenal medulla is under a direct nervous control, and the rate of its catechol secretion is primarily dependent on the impulse frequency in these secretory nerve fibres. There are, as mentioned, good reasons to assume that the rate of impulse discharge in sympathetic nerve fibres is reasonably low, probably never exceeding 10 impulses per second even at very strong reflex activation of the sympathetic nervous system. Therefore, electrical supramaximal stimulation of the sympathetic nerve fibres innervating the adrenals up to that rate will give some information concerning the upper limit of catechol release from the adrenals. However, it should be pointed out already at the outset that the upper limit thus obtained more is to be considered as the theoretical top capacity of the adrenals rather than representing what might be released into the blood stream upon various types of reflex excitation of the adrenals. This is partly due to the dual nature of the adrenal medullary gland cells.

Method

In this series there were no attempts to differentiate adrenaline and noradrenaline or to determine their relative proportions in the adrenal medullary output. Information on this matter regarding the cat will be found in e. g. the papers by Hökfelt (1951/52, 1953), Dunér (1953), Euler and Folkow (1953) and Hillarp and Hökfelt (1953). A quantitative parallel assay of adrenaline and noradrenaline necessitates a withdrawal of the adrenal venous outflow which is subsequently tested on its catechol content on special test preparations outside the animals body on which adrenaline and noradrenaline differ markedly in their actions. However, this interference with the blood supply and normal circulation through the adrenal

gland makes it difficult to be certain whether the collection of catechols was complete or not. In the present study it was considered more important to ascertain a reliable estimate of the total amount of catechols secreted rather than to get information about the relative proportions of adrenaline and noradrenaline. Therefore, the tests for the identification of catechols were chosen within the experimental animal itself, as in this case there will be no deviations from normal conditions regarding the circulation of the adrenals. The indicators for the identification of catechols available in the cat do not, however, differ sufficiently in their sensitivity towards adrenaline and noradrenaline to permit a reliable quantitative differentiation of these two catechols. Therefore, as long as the general aim was to determine the total amount of catechols released, without any further knowledge about their relative proportions, it was necessary to use test preparations on which adrenaline and noradrenaline have approximately the same degree of activity. The tests applied in this series were the denervated nictitating membrane of the cat (ROSENBLUETH and CANNON 1932) and the blood pressure of the spinal cat (see McDOWALL 1938) which both have been commonly used for the identification of catechols.

Operative procedures. Experiments were performed on thirty-two cats. The anaesthetic was chloralose (50 mg/kg) plus urethane (100 mg/kg) administered intravenously. A tracheal cannula was inserted and the abdomen was opened in the midline. In order to get free access to the left splanchnic nerves it was necessary to extirpate the intestines, the great omentum and the spleen. Another reason for this procedure was that stimulation of the splanchnic nerves will excite not only the secretory nerves to the adrenals but also vasoconstrictor nerve fibres to the splanchnic region. This will interfere with the blood-pressure responses induced by a release of catechols from the adrenal medulla. Therefore, in some animals, the sympathetic nerve fibres joining the renal vessels were severed in addition. These steps to a large extent reduced the splanchnic neurogenic vasoconstriction which otherwise would have accompanied the stimulation of the splanchnic nerves and to which the blood-pressure increase, at least partly, could be attributed.

Of the two adrenals the right one, and especially its secretory nerves, are not easy of access whereas the splanchnic nerves to the

left adrenal are easily isolated. Therefore, the right adrenal was excluded by ligatures. On the left side the greater and the lesser splanchnic nerves were isolated just below their entrance into the abdomen and centrally cut.

By a dentist's drill the spinal medulla was exposed from behind at the height of the sixth cervical segment and transected. Bleeding was controlled by cotton wool and gelatine sponge pellets. Artificial respiration was then applied and the rate and amplitude of the pump were adjusted to the spontaneous breathing that prevailed before the transection of the spinal medulla was performed. Both vagal nerves were cut in the neck. The arterial blood pressure was recorded by an ordinary mercury manometer connected to the left femoral artery.

The contractions of the left denervated nictitating membrane in response to the medullary catechols were recorded as described by ROSENBLUETH and CANNON (1932). In order to attain maximal sensitivity of the nictitating membrane the corresponding superior cervical sympathetic ganglion had been removed during aseptic conditions about two weeks prior to the experiment. To avoid mechanical interference with the contractions of the membrane the eyeball was emptied and the eyelids were incised and tied aside. By a silk thread the edge of the membrane was connected to a lever giving approximately a fifteenfold magnification of the contractions.

In the cat the greater and lesser splanchnic nerves form the major pathways of the secretory nerve fibres to the adrenals (HOLLINSHEAD 1936, YOUNG 1937/38, MAYCOCK and HESLOP 1939 and TEITELBAUM 1942). The greater splanchnic nerve is stated to carry the preponderance of these fibres. Additional fibres of minor importance are claimed to reach the adrenals from the upper two lumbar sympathetic ganglia (MAYCOCK and HESLOP 1939). In the present series the peripheral ends of the left splanchnic nerves were stimulated by means of a thyratron stimulator giving rectangular single shock stimuli of variable duration, strength and frequency. The strength and duration of the impulses were so adjusted that the nerve fibres should always be maximally excited. This could be tested by successively increasing the voltage up to a point beyond which there was no further increase of the medullary output, as revealed by the responses of the denervated nictitating membrane. A duration

of 5 to 10 msec and a voltage of 3 to 5 V generally proved sufficient.

Solutions of l-adrenalinehydrochloride and l-noradrenalinehydrochloride in Tyrode solution with pH adjusted to 7.3 were prepared and put on ice. *In the following all data concerning dosage of catechols injected or amounts that were released from the adrenals are expressed as salts.* All injections or infusions of catechols were performed in the left femoral vein.

In the first instance l-adrenaline and l-noradrenaline were injected intravenously at a dosage ranging from 0.05 to 5 μg. This was done to ascertain that at least one of the two tests revealed approximately the same degree of sensitivity towards these compounds. If this was not the case any further quantitative determination of the adrenal medullary output could not be undertaken because the relative proportions of the two catechols were not known.

The next step was to excite the left adrenal medulla through stimulation of the left splanchnic nerves. The frequencies most commonly employed varied from 5 up to 20 impulses per second. The splanchnic stimulation was continued for 5 or 10 seconds. These short periods were chosen because the stimulation had to be performed repeatedly, and if the stimulation periods were prolonged this might quite probably damage the nerve fibres, exhaust the cholinergic synaptic transmission or perhaps reduce the stores of catechols. The blood-pressure responses and the contractions of the denervated nictitating membrane thus obtained were matched as closely as possible by known amounts of l-adrenaline, l-noradrenaline or mixtures of both. These substances were administered intravenously at a constant rate and the injections were continued for a period that corresponded to that of the splanchnic nerve stimulation. Thus it was possible to estimate the total amount of catechols secreted by one adrenal when activated at a given frequency of splanchnic stimulation. From these data it was also possible to compute the amount released from one adrenal for each supramaximal single shock stimulus. If now the upper limit of impulse frequency in the sympathetic nerve fibres innervating the adrenal medulla is approximately 10 per second a general estimate of the theoretical maximum of catechol secretion from both adrenals could be achieved.

It might be questioned whether the right and left adrenal gland equalled in size. This matter could not be settled by weighing the glands subsequent to the experiments because the two glands had been handled quite differently during the experiment and could therefore not be compared. To get some information on this question in a control series of twenty-two cats both adrenals were extirpated, freed from connective tissue and weighed. These animals had been used in neurophysiological experiments and there was no interference with the adrenals which could have affected the two adrenals unequally. The difference in weight between the right and left adrenal did never exceed 20 per cent and these differences were quite irregular. There was thus no support of the view that e. g. the right adrenal should be regularly larger or smaller than the left one. This fact justifies the assumption that the total amount of catechols released from both adrenals corresponds approximately to double that determined for one adrenal gland only.

In a smaller series comprising six experiments another approach was tried. The splanchnic stimulation was then continued until the contraction of the nictitating membrane and the blood-pressure elevation reached a 'steady state'. These responses were then matched as closely as possible by intravenous infusions at a constant rate of l-adrenaline and l-noradrenaline.

Results

A distinct contraction of the denervated nictitating membrane was usually recorded in response to 0.1 to 0.2 μg l-adrenaline or l-noradrenaline. Amounts of about 5 μg quite regularly produced an almost maximal response. Doses between these limits provoked responses of the membrane which were graded to the amounts given.

Of the two catechols l-noradrenaline was slightly more effective on the denervated nictitating membrane than was l-adrenaline. The activity ratio for l-noradrenaline and l-adrenaline in this series of experiments taken as a whole was found to average 0.8/1. That is to say that if a given amount of l-adrenaline elicited a certain contraction of the membrane approximately the same effect was obtained in response to 80 per cent of that amount if l-noradrenaline was administered. This is the average figure and moderate variations occurred in different experiments. In a great number

of animals the denervated nictitating membrane was found to show about the same degree of sensitivity to l-adrenaline as to l-noradrenaline.

As to the effects on the blood pressure of the spinal cat the two catechols differed more markedly.

At a dosage of about 0.05 to 0.2 μg l-adrenaline quite often evoked a short fall of the blood pressure. This depressor capacity of adrenaline is most probably related to its well known dilator action on the blood vessels of skeletal muscles. If the dose was increased, e. g. up to 0.5 to 2 μg, the dilator action of adrenaline was still apparent from the characteristic downward 'notching' of the blood-pressure curve which was followed by a secondary rise. The blood-pressure response to still larger doses was an almost pure rise.

l-Noradrenaline, on the other hand, in all effective doses produced a blood-pressure rise. At a dosage of 0.2 to 0.5 this rise amounted to 20 to 40 mm Hg. The upper limit of the blood-pressure responses is probably determined by the efficiency of the heart, and when amounts exceeding 5 to 10 μg were given, the magnitude of the blood-pressure responses no longer properly run parallel with the amounts administered.

Thus, when comparing the pressor capacity of the two catechols noradrenaline was found to be the more effective. This was obviously the case when smaller amounts (e. g. up to 2 μg) were injected. However, in many experiments, when the 'adrenaline-notching' of the blood-pressure record was small or absent, the pressor action of l-adrenaline equalled or exceeded that of l-noradrenaline. Furthermore, when larger amounts (e. g. 2—10 μg) were administered the preponderance of l-noradrenaline regarding the pressor capacity was much less evident. It is not possible to present a reliable figure concerning the activity ratio for l-noradrenaline and l-adrenaline on the blood-pressure test, because this ratio was not fixed but differed according to the amounts administered.

As mentioned previously, the requisite must be set forth that at least one of the two tests applied should have approximately the same degree of sensitivity towards both catechols. Otherwise, the total amount of catechols released from the adrenal medulla upon splanchnic stimulation could not be determined unless the relative proportions of l-adrenaline and l-noradrenaline in the

medullary output were known. Regarding the denervated nictitating membrane this requisite was fulfilled in a large number of experiments and in many animals the two substances fairly accurately parallelled in their actions upon this test preparation. If in a single experiment the activity ratio between l-noradrenaline and l-adrenaline was below 0.8/1 or if it passed 1.2/1 the nictitating membrane was considered not to be sufficiently reliable to permit any further quantitative estimate. In other words, the contraction in response to a certain amount of l-adrenaline had to be matched by a dose of l-noradrenaline being approximately 80 to 120 per cent of that of l-adrenaline. In 14 out of the 32 experiments included in this series the activity ratio fell within the abovementioned limits. In this respect the blood-pressure test was less reliable and, as mentioned, the activity ratio between l-noradrenaline and l-adrenaline varied according to the magnitude of the doses given. Besides, the blood-pressure rise that accompanied the splanchnic stimulation was probably not entirely related to the actions of the catechols released from the adrenal medulla. They must also, at least in part, be attributed to a neurogenic vasoconstriction in the reduced splanchnic area. Therefore, the estimates of the catechol release from the left adrenal medulla were, in the first place, based upon the magnitude of the contractions of the denervated nictitating membrane. The blood-pressure test was more considered to furnish a general checking rather than to be conclusive regarding the magnitude of the medullary discharge.

Fig. 1 illustrates the procedure of these experiments. Of the two catechols l-noradrenaline was slightly more effective in raising the blood pressure in this animal than l-adrenaline. On the denervated nictitating membrane the two catechols run almost parallel in their actions at a dosage ranging from 0.2 to 2 μg. The catechols released from the left adrenal upon stimulation of the left splanchnic nerves during 10 seconds at a rate of 5 impulses per second (*E*) induced a contraction of the membrane that was somewhat more prolonged than those in response to 0.5 μg of l-noradrenaline (*F*) and l-adrenaline (*G*). On the other hand it was definitely smaller than the contractions following 1 μg of catechols (*C* and *D*). The response of the membrane accompanying stimulation of the left splanchnic nerves at 8 impulses per second was rather similar to

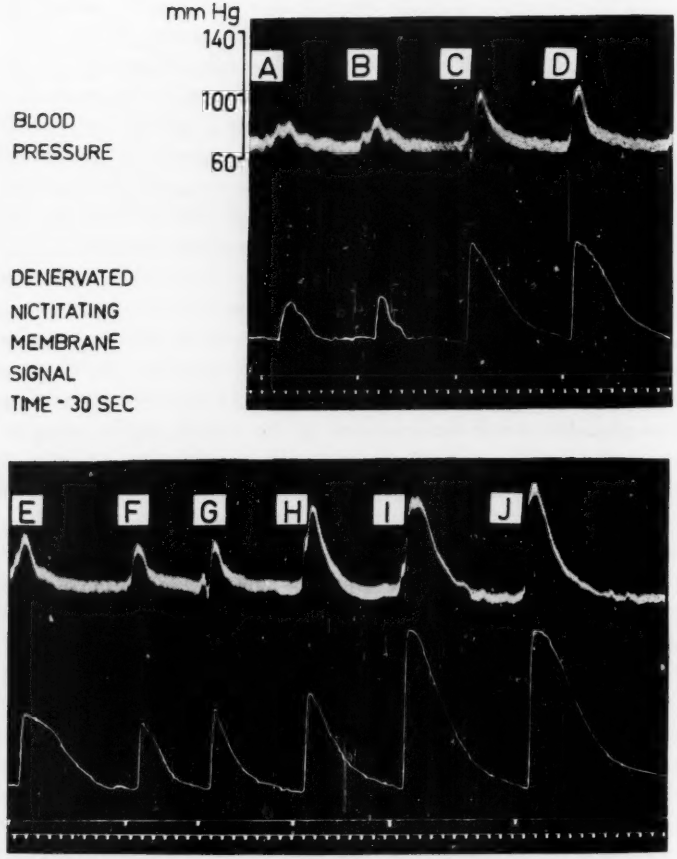

Fig. 1. Spinal cat, 2.8 kg. Determination of the rate of catechol secretion from the left adrenal. *A* — I. v. injection 0.2 μg l-adrenaline, *B* — 0.2 μg l-noradrenaline, *C* — 1 μg l-adrenaline, *D* — 1 μg l-noradrenaline, *E* — Stim. left splanchnic nerves (5/sec), *F* — 0.5 μg l-noradrenaline, *G* — 0.5 μg l-adrenaline, *H* — Stim. left splanchnic nerves (8/sec), *I* — 2 μg l-adrenaline, *J* — 2 μg l-noradrenaline. Stimulations and injections were all performed during 10 seconds.

TABLE 1. *The magnitude of the adrenal medullary discharge.*

No.	Body weight	Stimulation of the left splanchnic nerves		Amount of catechols released from left adrenal gland during period of stimulation	Amount of catechols released from left adrenal gland by each single shock stimulus	Calculated rate of catechol secretion from both adrenal glands when activated at 10 impulses per second
		Frequency	Period of stimulation			
	kg	impulses/sec	sec	μg	mμg	μg/kg/min
1	3.2	15	10	> 2.0 < 3.0	> 13 < 20	> 5.0 < 7.5
2	4.8	15	5	> 1.0 < 1.5	> 13 < 20	> 3.3 < 5.0
3[1])	2.8	8	10	> 0.5 < 1.0	> 6 < 13	> 2.7 < 5.4
4	4.3	20	5	> 1.5 < 2.0	> 15 < 20	> 4.2 < 5.6
5	3.5	16	10	> 2.0 < 3.0	> 13 < 19	> 4.3 < 6.4
6	4.8	10	5	> 1.0 < 1.5	> 20 < 30	> 5.0 < 7.5
7	3.6	10	10	> 2.0 < 3.0	> 20 < 30	> 6.7 < 10.0
8	3.3	8	10	> 1.0 < 1.5	> 13 < 19	> 4.5 < 6.8
9	3.5	6	10	> 1.0 < 1.5	> 17 < 25	> 5.7 < 8.6
10	3.3	20	10	> 1.0 < 2.0	> 5 < 10	> 1.8 < 3.6
11	2.0	20	10	> 1.0 < 2.0	> 5 < 10	> 3.0 < 6.0
12	3.2	10	10	> 1.0 < 1.5	> 10 < 15	> 3.8 < 5.6
13	3.8	15	10	> 1.0 < 2.0	> 7 < 13	> 2.1 < 4.2
14	2.6	10	10	> 1.0 < 1.5	> 10 < 15	> 4.6 < 6.9

[1]) see fig. 1.

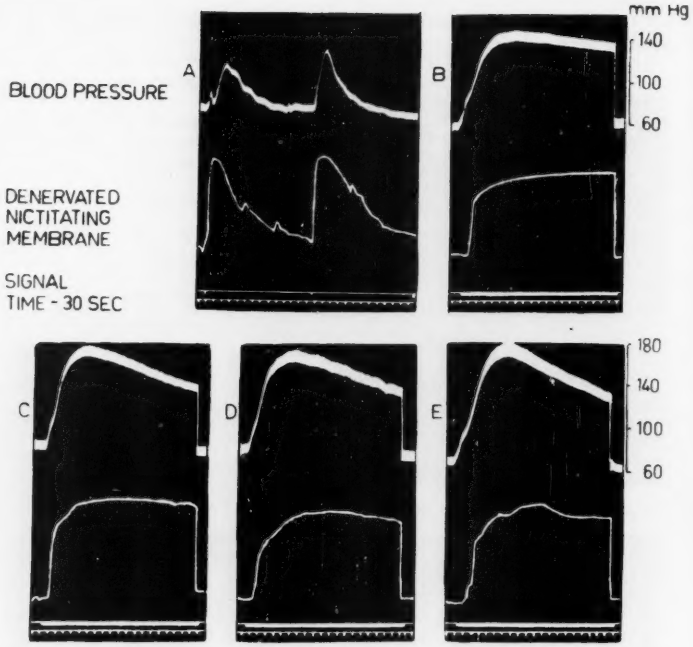

Fig. 2. Spinal cat, 3.1 kg. Determination of the rate of catechol secretion from the left adrenal. *A* — 1 μg l-adrenaline, 1 μg l-noradrenaline, *B* — Stim. left splanchnic nerves (4/sec), *C* — I. v. infusion of a mixture of l-adrenaline (30 per cent) and l-noradrenaline (70 per cent) — in total 1.8 μg/kg/min, *D* — Infusion of catechols — in total 1.2 μg/kg/min, *E* — Stim. left splanchnic nerves (6/sec). Kymograph arrested when stimulations and infusions were interrupted.

those effected by 1 μg of the catechols although, maybe, of a somewhat shorter duration. Thus it might be claimed that the amount of catechols that was released from the left adrenal when excited at a frequency of 8 impulses per second during 10 seconds was larger than 0.5 μg but at the same time did not exceed 1 μg. It was probably closer to the latter figure than the former.

In this series of experiment it was further noticed that when very short periods of left splanchnic nerve stimulation were employed (5 or 10 seconds) the amount of catechols released was roughly a linear function of the frequency applied up to approximately 20 impulses per second. When the left splanchnic nerve stimulation

was continued for several minutes at frequencies higher than about 15 per second there was, however, a progressive deterioration of the adrenal medullary discharge.

The experiments in this series are summarized in table 1. It is obvious that in a biological assay of this type it is hardly possible to present any exact figure on the catechol release. All the actual figures given are just limits within which the true amount should be found. On the basis of the assays in this study it is claimed that when *all* cells of the adrenal medullae are activated at the impulse frequency of 10 per second the catechol secretion in the cat as a rough general average is 5 μg/kg/min.

The complementary study where the stimulation of the left adrenal and the infusion of catechols were continued until the contraction of the membrane reached a 'steady state' is illustrated by fig. 2. As seen from this fig. the contraction of the denervated nictitating membrane following left splanchnic nerve stimulation at 4 per second (*B*) is rather similar to that effected by infusion of catechols at a rate of 1.2 μg/kg/min (*D*). Likewise *E* (6/sec) rather well equals *C* (1.8 μg/kg/min). Accordingly, in this experiment the catechol secretion from both adrenals when activated at 10 impulses per second can be calculated to approximate 6 μg/kg/min.

Comments

The adrenal medullary secretion of the cat in response to splanchnic nerve stimulation at different frequencies has previously been investigated by ROSENBLUETH (1932). He stated that for each stimulus the amount released from one adrenal was 4.8 mμg of 'adrenaline'. That is a considerably lower figure than was found in most of the experiments in the present series (see table 1). The figure reported by ROSENBLUETH refers to a single experiment but was said to coincide with 'the average of three observations on different animals'. Obviously these few experiments are not conclusive. Besides, the fact that noradrenaline constitutes a major fraction of the medullary discharge was not realised in 1932. The sensitivity of the tests applied by ROSENBLUETH might differ regarding adrenaline and noradrenaline and therefore any quantitation of the medullary output in terms of adrenaline is not reliable.

The upper limit of catechol secretion from the adrenals in the present series of experiments was claimed to average some 5 μg/kg/min when all cells were concomitantly activated at the top of the physiological discharge range of sympathetic nerve fibres. This upper limit is considerably higher than the rate of catechol secretion that has been observed in the cat during various types of reflex activation (for references see e. g. CANNON and ROSENBLUETH 1937, MCDOWALL 1938, ROSENBLUETH 1950, DUNÉR 1953 and EULER and FOLKOW 1953). It rather represents the theoretical average upper limit of catechol secretion in the cat which will probably never be reached during any type of reflex activation of the adrenal medullae in the living body. However, a general estimate of this upper limit gives some information about the amounts of catechols which with some justification might be called 'physiological'.

It was found by EULER and FOLKOW (1953) that in the cat the composition of the adrenal medullary output following left splanchnic nerve stimulation varied within rather wide limits regarding the relative proportions of the two catechols. Thus the percentage of noradrenaline ranged from 55 to 93 per cent of the total amount of catechols released (mean average of 9 experiments was 73 per cent). In the following comparative study on the relative effectiveness of the direct sympathetic innervation and the adrenal medullary discharge on various smooth muscle effectors (Chapter III) the actual proportions of adrenaline and noradrenaline might then differ widely in different experiments. A complementary investigation on the effects of adrenaline and noradrenaline on these effectors was therefore included in this comparative study, and the catechols were infused separately in increasing amounts up to approximately 5 μg/kg/min.

CHAPTER III

The control of some smooth muscle effector groups by the sympathetic innervation and the adrenal medullae

The purpose of the present study was to map out the range of control — especially of some vascular regions — exerted by the direct sympathetic innervation when activated by maximal electrical stimulation at various frequencies within the physiological discharge range as compared with that of the adrenal medullary hormones released by electrical stimulation of the innervation to the adrenal glands. Further, the dose-response curves of l-adrenaline and l-noradrenaline on the same effector groups were determined.

A. The blood vessels of skeletal muscles

Apart from the work by FOLKOW (1951, 1952) and CELANDER and FOLKOW (1953) there is practically nothing known about the range of control of blood vessels of skeletal muscles by way of the sympathetic vasoconstrictor nerves. The justification for including a similar study in this work is that the effects produced by vasomotor nerve stimulation and those of the adrenal medullary discharge had to be quantitatively compared in the same animal. The action of the adrenal medullary hormones on blood vessels of skeletal muscles has been reviewed by MCDOWALL (1938) and recently by BARCROFT and SWAN (1953). But to my knowledge there is no actual quantitative comparison of the range of control of the adrenergic constrictor fibres on the one hand and of the humoral control by the adrenal medullae on the other. Such a comparison should be carried out with a good method to record blood flow. Further, the activation of the constrictor nerves as well the secretory nerves to the adrenals should be performed at known frequencies within the physiological discharge range of the sympathetic nervous system.

Method

Experiments were performed on twenty-two cats anaesthetized with chloralose (50 mg/kg) and urethane (100 mg/kg). The abdomen was opened and the intestines were extirpated in order to get free access to the splanchnic nerves and the abdominal sympathetic chains. Care was always taken to leave the arterial supply of the liver intact, which is necessary in order to keep the animals in a good condition. To avoid any reflex discharge of adrenaline and noradrenaline from the adrenal glands the left one was sympathetically decentralized and the right one extirpated. The peripheral ends of the left splanchnic nerves were isolated for subsequent stimulation.

At the height of the fourth and fifth lumbar vertebrae the right sympathetic chain was carefully isolated and later on cut just below the fourth lumbar sympathetic ganglion. The influence from the vasomotor centre on the vessels of the hind limb was consequently interrupted, while at the same time it was quite possible to activate practically all the sympathetic vasomotor nerves to the vessels by electrical stimulation of the peripheral end of the sympathetic chain.

The right hind limb was carefully skinned and the circulation through the paw was interrupted by a tight ligature around the ankle. To avoid cooling and drying of the muscles, the skinned limb was covered with gauze moistened with warm Tyrode solution and the skin was reapplied around the muscles. The femoral vein, which is the major drainage of the lower part of the skinned hind limb, was isolated and cannulated just proximal to the entrance of the popliteal vein. By way of a polyethylene tube the venous outflow was directed to an optical 'drop recorder' operating an 'ordinate writer' (Clementz and Ryberg 1949). This apparatus is so designed that within a rather wide range of drop flows the ordinates are approximately inversely proportional to the rate of blood flow.

By a continuous drop infusion into one of the external jugular veins the blood, after passing the recording set, was returned to the animal and the inflow rate was cautiously adjusted to the outflow rate. In these and subsequent experiments heparin was regularly administered to prevent blood clotting.

The variations in blood pressure which accompanied the vasomotor nerve stimulation or the infusion of l-adrenaline or l-noradrenaline

and which implied disturbing changes of the perfusing pressure to the vascular region investigated, were eliminated by continuously adjusting a screw clamp applied around the lower abdominal aorta. The guide followed was the arterial blood pressure in the left femoral artery recorded by an ordinary mercury manometer. The screw clamp on the aorta was adjusted so that this pressure, and consequently the perfusing pressure to the skinned right hind limb, remained unaltered in spite of large variations in systemic arterial blood pressure. Thus, haemodynamic effects caused by hydrostatic factors were eliminated and changes of blood flow could safely be related to the vasomotor nerve stimulation or to the action of l-adrenaline and l-noradrenaline, released from the left adrenal by splanchnic stimulation or infused into the blood stream at a constant rate.

The stimulation of the sympathetic vasomotor fibres to the blood vessels of the hind limb and of the splanchnic nerves to the left adrenal was performed by means of thyratron stimulator giving rectangular single shock stimuli of variable duration, intensity and frequency. A voltage of 3 to 5 V and a duration of impulse of 5 to 10 msec produced a maximal excitation in most animals.

In order to block the interfering activity of cholinergic vasodilator fibres within the abdominal sympathetic trunks, and which are always concomitantly excited upon stimulation, atropine at a dosage of 0.2 to 0.3 mg/kg of body weight was given intravenously. This amount has been found sufficient to block these dilator fibres in most animals whereas the adrenergic constrictor fibres are not affected (Eliasson, Folkow, Lindgren and Uvnäs 1951, Folkow 1952 and Folkow and Gernandt 1952).

Intravenous infusions of l-adrenaline and l-noradrenaline were done through the left brachial vein and intraarterial infusions through the central stump of the inferior mesenteric artery.

Results

Stimulation of sympathetic vasoconstrictor fibres to the blood vessels of skeletal muscles

As long as the perfusion pressure is constant the heights of the ordinates in the record are approximately directly proportional to the peripheral resistance of the vascular region investigated. The

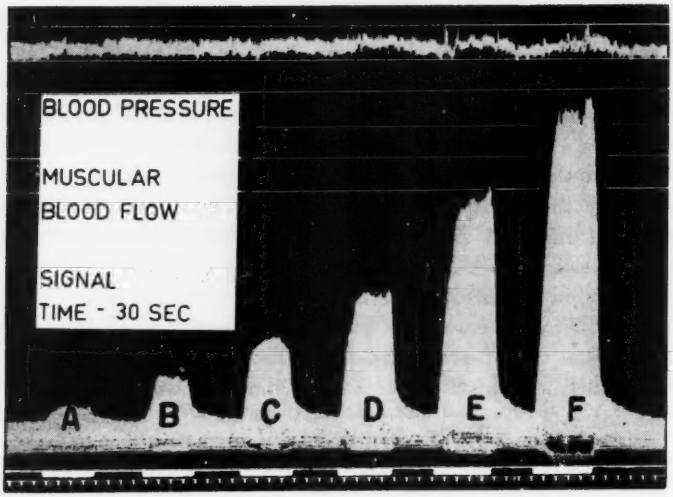

Fig. 3. Blood flow of a muscular vascular region. Effects of vasoconstrictor nerve stimulation. 'Perfusing' blood pressure 120 mm Hg. As long as the 'perfusing' blood pressure remains constant the heights of the ordinates in the record are approximately directly proportional to regional resistance towards blood flow. *A* — Stim. lower abdominal sympathetic trunk (30/min), *B* — Do. (1/sec), *C* — Do. (2/sec), *D* — Do. (4/sec), *E* — Do. (6/sec), F — Do. (10/sec).

fact that the vessels of the muscles are no longer under the control of the vasomotor centre does not imply that they therefore are maximally dilated. On the contrary they exhibit a rather marked tone which most probably is due to an inherent property of the smooth muscle cells of the blood vessels and might well be called 'myogenic'. The strongest vasoconstriction obtained corresponded to an increase of peripheral resistance of maximally some 10—12 times. These effects were reached at frequencies about 8—15 impulses per second and were not augmented by any further increase of stimulation rate. Even at very low frequencies the increase of peripheral resistance is quite obvious with a corresponding reduction of blood flow. At a frequency of one impulse every other second to one impulse per second the characteristic finding was about a twofold increase of peripheral resistance. These effects are illustrated by fig. 3.

The adrenal medullary discharge on the blood vessels of skeletal muscles

This study was performed on the sympathetically decentralized vascular region of the skinned hind limb. Any hydrostatic interference and reflexly induced release of catechols from the adrenals were eliminated as previously mentioned. The left adrenal medulla was activated by way of maximal electrical stimulation of the left splanchnic nerves at different frequencies. Consequently, the changes of blood flow in the skeletal muscular region must be attributed to the hormones released from the adrenal medulla.

Adrenaline is since long known to dilate skeletal muscle blood vessels, especially when administered in small amounts, while larger doses bring about a vasoconstriction. The type of response is, however, not only a matter of dosage but also intimately related to the prevailing state of the vessels as it is influenced by the experimental conditions. Sympathetically decentralized vessels will dilate to some degree due to the elimination of a tonic constrictor fibre discharge and the general condition of the animal will further influence the tone. It is quite obvious that the lower the prevailing vascular tone is, the less valid is the preparation for the evaluation of a possible dilator action of any compound. To test the 'inherent' tone a supramaximal dose of acetylcholine was given by 'close arterial' injection. The magnitude of the maximal dilatation thus obtained will give some information about the 'basal' tone of the vessels in the region studied.

Fig. 4 illustrates the characteristic findings when the left adrenal medulla was activated by splanchnic nerve stimulation at different frequencies. At *A* 20 μg acetylcholine was given as a 'close arterial' injection to the vessels of the muscles of the hind limb. After 10—15 seconds a maximal dilator response was reached with a sixfold increase of blood flow as compared with the rate of flow prior to the injection. This dilatation was a maximal one, because larger amounts of acetylcholine did not dilate the vessels any further, and therefore denotes the minimal resistance to blood flow which the muscular vessels could show. Further, this pronounced dilatation in response to acetylcholine indicates that the denervated vascular region had a good 'basal' tone.

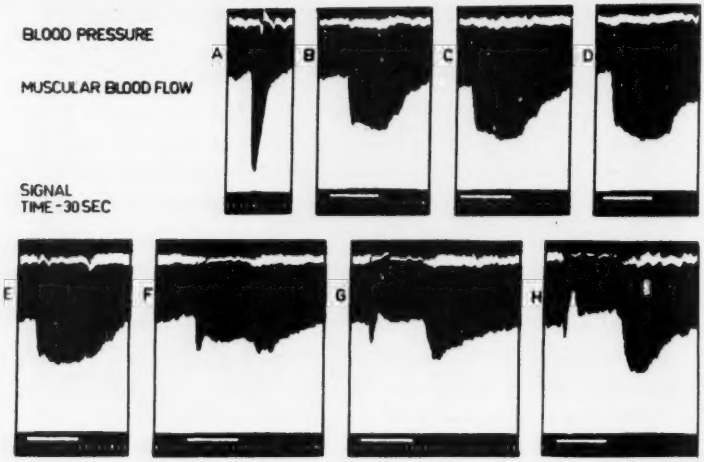

Fig. 4. Effects of adrenal medullary discharge on muscular blood flow. 'Perfusing' blood pressure 120 mm Hg. *A* — 'Close-arterial' injection 20 μg acetylcholine, *B* — Stim. left splanchnic nerves (1/sec), *C* — Do. (2/sec), *D* — Do. (4/sec), *E* — Do. (6/sec), *F* — Do. (10/sec), *G* — Do. (14/sec), H — Do. (20/sec).

B-H of the same record show the responses of the blood vessels to the discharge from the left adrenal gland induced by maximal splanchnic stimulation at various frequencies. A dilatation was obtained which persisted as long as the splanchnic stimulation was continued. This dilatation was pronounced already at low stimulation frequencies, reaching a maximum at 4 impulses per second (*D*). Higher frequencies reduced this dilatation but did not turn it into a vasoconstriction. Subsequent to the periods of splanchnic stimulation at the higher frequency range (*G-H*) there was a secondary vasodilatation which in some animals was quite pronounced and was maintained for some minutes.

The dilator response to left splanchnic nerve stimulation was a quite common observation as long as the 'basal' tone of the blood vessels remained at a high level. In some animals it was not as pronounced as in the experiment referred to in fig. 4, and in others stimulation at higher rates (exceeding approximately 8 per second) induced a moderate vasoconstriction reducing blood flow to about half the initial rate. However, in most of these experiments the

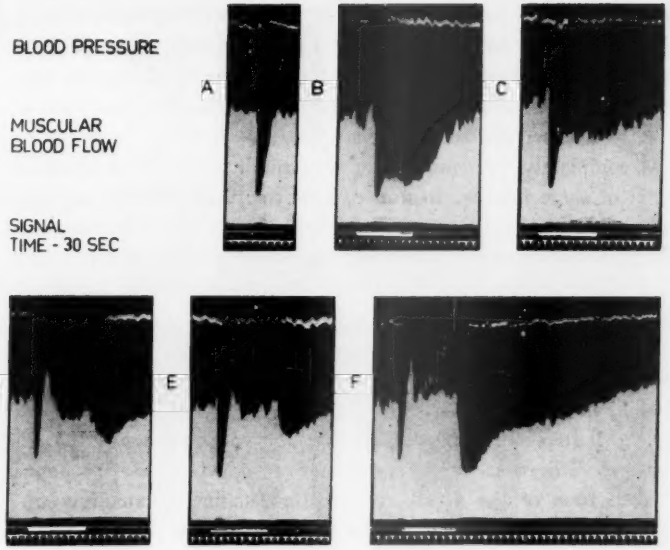

Fig. 5. Effects of l-adrenaline on muscular blood flow. 'Perfusing' blood pressure 120 mm Hg. *A* — 'Close-arterial' injection 20 μg acetylcholine, *B* — I. v. infusion l-adrenaline (0.3 μg/kg/min), *C* — Do. (0.55 μg/kg/min), *D* — Do. (1 μg/kg/min), *E* — Do. (1.9 μg/kg/min), *F* — Do. (3.5 μg/kg/min).

acetylcholine test revealed a more or less reduced 'basal' tone. It must also be remembered that the relative percentage of adrenaline and noradrenaline in the medullary discharge varies considerably from animal to animal.

Intravenous infusion of l-adrenaline

At *A* in fig. 5 20 μg acetylcholine was given by the inferior mesenteric artery. After 10—15 seconds there was a fivefold increase of blood flow which reveals that the peripheral resistance at maximal dilatation was only about one fifth of that at the 'basal' tone equilibrium. Larger amounts of acetylcholine did not increase this response. *B-F* of the same record demonstrate the dilator action of l-adrenaline given by continuous intravenous infusion at a constant rate at a dosage ranging from 0.3 to 3.5 μg/kg/min. The initial vasodilatation at *B* was a maximal one and it was followed

by a sustained but somewhat less pronounced vasodilatation for the rest of the infusion. During this later period the peripheral resistance was reduced to about forty per cent of control value. When larger amounts were infused this sustained vasodilatation, and to a lesser degree the initial vasodilatation too, were more or less completely overcome by a constrictor action of l-adrenaline. It is of some interest to notice, however, that amounts up to 3.5 μg/kg/min as a *net* result did not bring about a constriction of the skeletal muscle vessels. Another interesting point is the vasodilatation which appeared and lasted well after the infusion of l-adrenaline had been interrupted. It is noticeable at *D* (1 μg/kg/min) and is quite marked at *F* (3.5 μg/kg/min) where it is effected by a decrease in peripheral resistance to about forty per cent of the initial level. For further discussion on this matter, see the following 'Comments' (p. 46). In those animals in which for various reasons the 'close arterial' injection of acetylcholine revealed a less pronounced 'basal' tone of the small vessels, l-adrenaline in amounts up to 2 μg/kg/min still elicited a dilatation which was almost maximal. Larger amounts changed the response into a vasoconstriction which, however, in amounts not exceeding 5 μg/kg/min was moderate and seldom decreased blood flow more than about fifty per cent. It is probable, however, that such preparations do not picture properly the balance between the dilator and constrictor action of l-adrenaline, when, already at the beginning, the small vessels are almost maximally dilated. A pronounced 'basal' tone of the vessels in skeletal muscles is a quite regular finding in the cat and was also observed in man by Barcroft, Bonnar, Edholm and Effron (1943). It can therefore not be looked upon as some experimental 'artifact' but rather as representing the true condition.

Intravenous infusion of l-noradrenaline

The changes of blood flow are illustrated by fig. 6. At *A* 20 μg of acetylcholine was injected in the central stump of the inferior mesenteric artery. The effect was a fourfold increase of blood flow showing that the 'basal' tone was rather good. *B-H* in this record show the effects of l-noradrenaline given by intravenous infusion at a constant rate for periods of four minutes. At *B* 0.1 μg/kg/min was

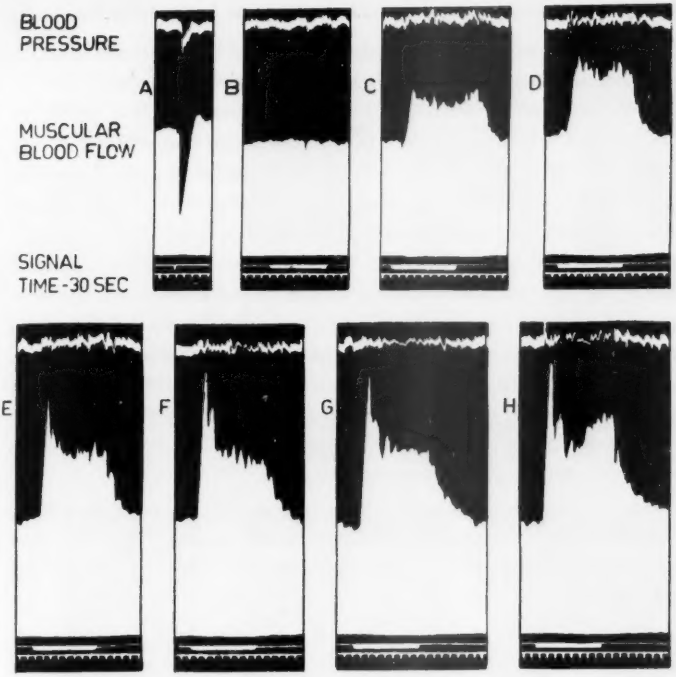

Fig. 6. Effects of l-noradrenaline on muscular blood flow. 'Perfusing' blood pressure 120 mm Hg. *A* — 'Close-arterial' injection 20 μg acetylcholine, *B* — I. v. infusion l-noradrenaline (0.1 μg/kg/min), *C* — Do. (0.2 μg/kg/min), *D* — Do. (0.4 μg/kg/min), *E* — Do. (0.7 μg/kg/min), *F* — Do. (1.2 μg/kg/min), *G* — Do. (2.1 μg/kg/min), *H* — Do. (3.8 μg/kg/min).

infused. In most animals amounts up to that dose did not affect the vessels. In no case was a dilatation obtained. Larger amounts evoked a pure vasoconstriction which, however, never was very impressive; the maximal effect demonstrating approximately a three-fold increase of peripheral resistance. Another point of some interest is the fact, which could be repeatedly demonstrated, that the initial vasoconstriction was not sustained but rapidly declined when giving larger amounts. See *E-H* in the same record. Further it was not followed by any 'after dilatation' as was the case with the infusion of l-adrenaline.

Intraarterial infusion of l-adrenaline and l-noradrenaline

The pronounced dilatation of skeletal muscle blood vessels brought about by low frequency stimulation of the adrenal medulla and by small amounts of l-adrenaline, infused intravenously, is of considerable interest when evaluating the physiological importance of the adrenal medulla. The mechanism of this dilatation is not of nervous origin because it occurred in vessels totally deprived of connections with the central nervous system. It might be a local affair due to (i) the direct action of adrenaline on the smooth muscle cells in the blood vessels, or (ii) related to some action of adrenaline on the surrounding skeletal muscle cells. It might also be due to (iii) the action of some vasoactive substance released by adrenaline somewhere else in the body. In order to test the relevance of this third possibility the administration of adrenaline had to be restricted to the vascular region investigated. This can be achieved by a 'close arterial' infusion but make certain precautions necessary.

For intraarterial infusions the central stump of the inferior mesenteric artery was cannulated. All vessels leaving the aorta below this branch except the two femoral arteries were ligated. As the left femoral artery was connected to the blood-pressure recording set the only pathway left for the infused substances was the right femoral artery. During the relatively brief periods of infusion the venous outflow from the cannulated femoral vein was *not* reinfused into the animal so that any amount of adrenaline, remaining in the venous blood, should not be able to reach the systemic circulation releasing any possible secondary factors. When the infusion periods were prolonged the blood volume was kept constant by slow intravenous administration of Dextrane-Tyrode solution to replace the venous outflow not reinfused. If the dilator response is still obtainable and unchanged in magnitude by 'close arterial' injection of l-adrenaline, it must be related purely to a direct action on smooth muscle cells in the blood vessels or to some change induced by l-adrenaline in the surrounding skeletal muscle cells. On the other hand, if there is no vasodilatation or, if the response is more or less decreased, the reason must be that some vasoactive humoral substance or substances released in other parts of the organism are more or less responsible for the vasodilator response in skeletal muscles.

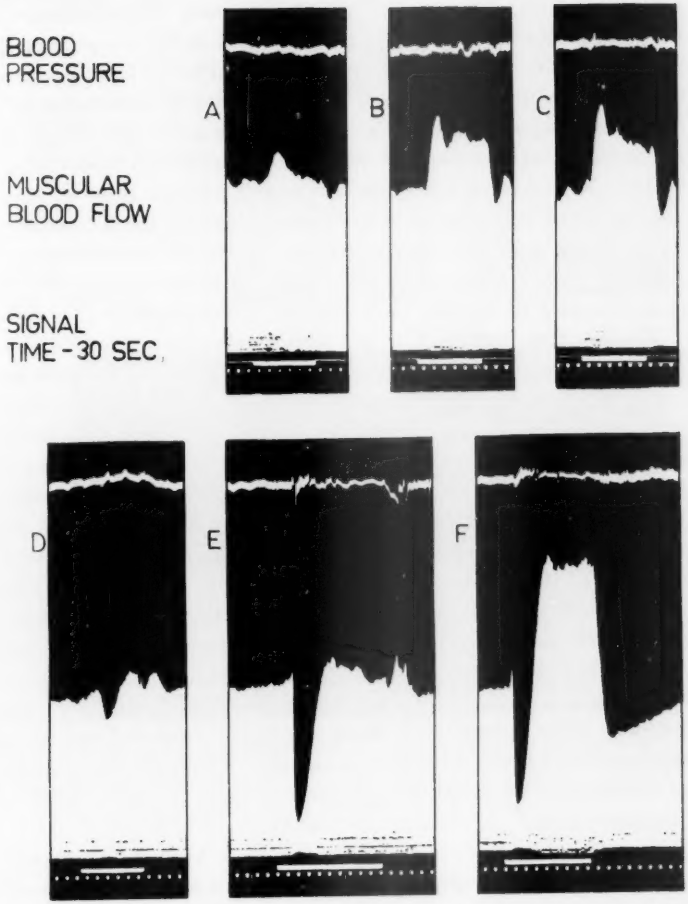

Fig. 7. Effects on muscular blood flow of 1-adrenaline and 1-noradrenaline given intraarterially. 'Perfusing' blood pressure 120 mm Hg. *A* — I. a. infusion 1-noradrenaline (0.07 μg/kg/min), *B* — Do. (0.12 μg/kg/min), *C* — Do. (0.21 μg/kg/min), *D* — I. a. infusion 1-adrenaline (0.07 μg/kg/min), *E* — Do. (0.12 μg/kg/min), *F* — Do. (0.21 μg/kg/min).

Fig. 7 shows the results in a representative experiment. 1-Adrenaline is still quite capable of dilating muscular blood vessels. At *E* the amount infused intraarterially was 0.07 μg/kg/min. At the beginning the vasodilatation was maximal and caused a fall in peripheral resistance to about twenty per cent of the initial level. The dilatation was, however, not sustained and larger amounts of 1-adrenaline (*F*) turned the response into a constriction during which the peripheral resistance was almost doubled. Still, 1-noradrenaline (*A-C*) did never in any concentration bring about a vasodilatation. It is of some interest that 1-adrenaline in larger doses, in spite of the competition with its now masked dilator effect, had a stronger vasoconstrictor capacity than 1-noradrenaline. Compare in fig. 7 *C* and *F* where equal amounts of the two catechols were administered.

Whether the constrictor or the dilator action of adrenaline predominates is basically a matter of dosage. This fact is illustrated by fig. 8 which shows that 1-adrenaline when infused intraarterially in minute amounts is quite capable of inducing a sustained vasodilatation. At *C* the Tyrode solution infused intraarterially contained 0.5 μg/ml. and the rate of infusion was 0.34 ml./min. After 10 seconds the dilatation started and it was maximal after an additional 10 seconds. Up to this moment the total amount of 1-adrenaline that had entered the blood stream to the muscles did not exceed 0.06 μg and, considering the circulation time from the point of infusion to the effector cells, the latency of the smooth muscle cells and the lag of the venous outflow, the true amount of 1-adrenaline that induced this initial maximal increase of blood flow was probably even smaller. A maximal dilator response was thus obtained by a minute amount of 1-adrenaline. As long as the infusion of 1-adrenaline was continued the vasodilatation persisted. At *A* in the same record (fig. 8) the dose was about half that at *C* and no change of blood flow occurred. On the other hand if the amount infused was doubled (*B*) the initial dilatation was rapidly turned into a moderate constriction. This is somewhat contrasting to the responses of intravenous infusion of 1-adrenaline where the dilatation persisted over a much wider range of dosage. A possible explanation is that the local dilator action of 1-adrenaline might be more or less heavily reinforced by some vasoactive substances liberated in the body, as e. g. lactic acid mobilized from *other* skeletal

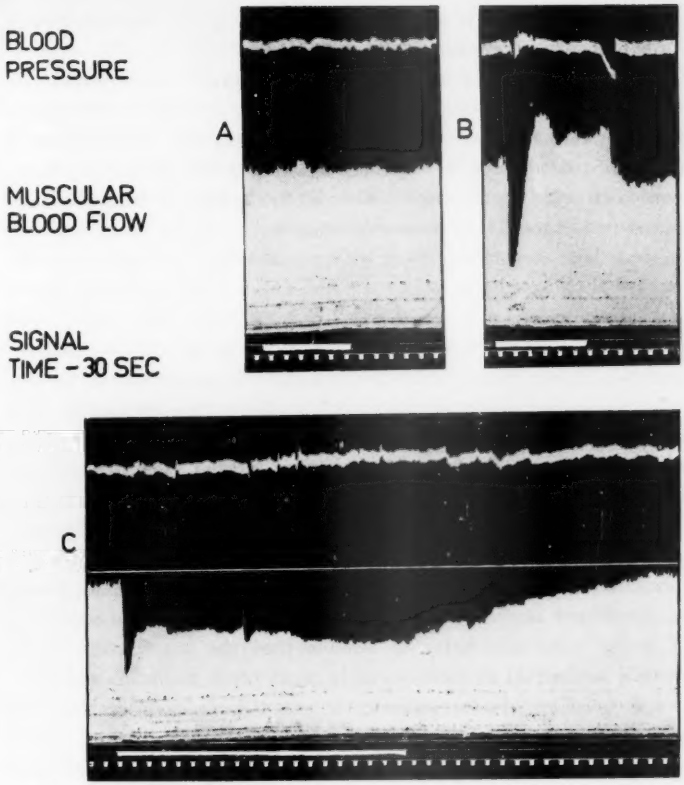

Fig. 8. Effects on muscular blood flow of l-adrenaline given intraarterially. 'Perfusing' blood pressure 120 mm Hg. Body weight 2.5 kg. *A* — I. a. infusion l-adrenaline (0.04 μg/kg/min), *B* — Do. (0.13 μg/kg/min), *C* — Do. (0.07 μg/kg/min). Note the sustained dilatation at C.

muscle regions, and probably attributed to the action of the intravenously administered l-adrenaline. It should be remembered that the skeletal muscle region investigated is in a good position to reveal the action of dilator compounds (as e. g. lactic acid) that may circulate in the blood. This region is disconnected from the central control at higher levels. In muscular regions with the vasomotor innervation intact the dilatator response to l-adrenaline may be

more or less completely masked by a neurogenic vasoconstriction induced by baroreceptor activities.

These experiments present some rather clear-cut evidence that the mechanisms behind the 'initial' vasodilatation, the 'sustained' dilatation as well as the vasoconstriction elicited by l-adrenaline on muscular blood vessels all must be looked for primarily in the skeletal muscle region itself. The dilator action of l-adrenaline is not dependent on the vasomotor nerves.

Comments

The action of adrenaline on the blood flow through an isolated skeletal muscle region is a complex one. If larger amounts were infused — especially by an intraarterial route — the vessels constricted and the peripheral resistance rose. It was demonstrated in the present study that, when the two catechols were infused 'close-arterially' in larger amounts to the muscular blood vessels, the constrictor effect of adrenaline is not less than that of noradrenaline (compare for instance *F* and *C* in fig. 7 where equal amounts of the two catechols were administered). As the skeletal muscle cells themselves are not known to release any vasopressor substances under the influence of adrenaline it seems quite reasonable to assume that the constriction of the vessels in skeletal muscles — as in most other vascular regions — is a *direct* effect of adrenaline on the *smooth muscle cells* in the blood vessel walls.

However, this constrictor effect of adrenaline is not very prominent until a fairly large amount is given. In any case, it seems to be more or less completely masked by the intense vasodilatation elicited by adrenaline which appears already at a far lower dosage. This dilator action, which in many animals resulted in a maximal dilatation, puts adrenaline in the first line among the substances known to dilate the blood vessels of skeletal muscles. The mechanism behind this dilatation is not settled. During recent years this problem has been investigated on man by Allen, Barcroft and Edholm (1946), Duff and Swan (1951) and Whelan (1952). Their results are summarized and discussed at length in a monograph by Barcroft and Swan (1953). Their main results on this subject are the following. Intravenous infusion of small amounts of adrenaline

causes a great initial increase in the blood flow of the forearm or calf in man. This initial vasodilatation of skeletal muscle blood vessels was transient and was followed by a less marked but sustained vasodilatation for the rest of the period of adrenaline infusion. The initial, as well as the sustained vasodilatation in muscular regions could be demonstrated in patients subject to sympathectomy, and thus were not dependent on the vasomotor nerve supply. However, only the initial transient increase of blood flow and not the sustained one was obtained if adrenaline were given by intraarterial infusion (Duff and Swan 1951). This dilator action of adrenaline is evident only when small amounts are administered. Larger doses will constrict blood vessels in skeletal muscles. Noradrenaline in all effective doses has a pure constrictor action on the small vessels in skeletal muscles whether given by an intravenous or an intraarterial route. For the literature on this matter see Barcroft and Swan (1953).

In the cat, however, as demonstrated in the present study, adrenaline, when acting strictly locally, is quite capable of effecting a sustained vasodilation though, maybe, not as pronounced as during intravenous infusions. Consequently the mechanism behind this dilatation must be looked for primarily in the skeletal muscle region itself.

It is hard to conceive that the *direct* effect of l-adrenaline on the smooth muscle cells of the muscular blood vessels at a low dosage should be a relaxation while the same substance on the same substrate at a higher dosage would bring about a constriction. It seems more reasonable to assume that the dilator action of l-adrenaline on these blood vessels is an *indirect* one and that its disappearance at a higher dosage of l-adrenaline is related to the *direct* constrictor action of l-adrenaline. In that case the dilatation would be due to the mobilization of a vasodilator factor released by l-adrenaline in the surrounding skeletal muscle cells with a secondary influence on the smooth muscles of the blood vessels.

The capacity of adrenaline to increase the rate of lactic acid formation in skeletal muscles is a well proven fact (see e. g. Cori, Cori and Buchwald 1930, Griffith, Lockwood and Emery 1939, Lundholm 1949 b and Griffith 1951). In cats, the minimal effective dose of adrenaline given by intravenous infusion was stated to

approximate 1 μg/kg/min (GRIFFITH et al. 1939). This threshold is about 10—20 times higher than that reported by CORI et al. (1930) and LUNDHOLM (1949) working on rabbits. The latter author presented evidence to prove that the increase of lactic acid content of the blood in the rabbit was maximal already at a dosage of 0.2 μg/kg/min. BEARN, BILLING and SHERLOCK (1951) stated that adrenaline administered intravenously at a rate of 0.1 μg/kg/min to normal unanaesthetized human subjects caused a considerable rise of the lactic acid content in peripheral venous blood while noradrenaline had little effect even if the rate of infusion was doubled. The glycogen stores of skeletal muscles in man was reduced by the action of adrenaline (HILDES, SHERLOCK and WALSHE 1949). The dilator action of lactic acid is a well known fact (for ref. see e. g. MCDOWALL 1938).

All these facts suggest that the vasodilatation of muscular blood vessels might be related to the glycogenolytic power of adrenaline, as was originally suggested by LUNDHOLM (1951). The increase of lactic acid production as well as the vasodilatation are both pronounced at a rather low dosage of l-adrenaline which seems to be definitely lower than the dosage which induces constriction of smooth muscles. On the other hand, the lactacidaemic action of l-noradrenaline (BEARN et. al. 1951) is small as compared with its vasoconstrictor capacity, and in the present study l-noradrenaline was never observed to dilate skeletal muscle vessels. It is important to stress that if the mechanism postulated above is the true one, *the dilatation of blood vessels in the skeletal muscles is to be considered a phenomenon secondary to changes in the carbohydrate metabolism of skeletal muscles induced by adrenaline, and therefore should be looked upon more as a 'metabolic' action of adrenaline rather than a direct 'motor' action.*

The 'after-dilatation' which appeared when the infusion of adrenaline was interrupted and which sometimes was quite impressive, especially when larger amounts of l-adrenaline were given intravenously (see fig. 5), is somewhat obscure. It cannot be looked upon as a true 'reactive hyperaemia' because in most animals adrenaline in the given concentration did not decrease the blood flow through the skeletal muscles. No 'after-dilatation' was seen to follow upon the constriction produced by noradrenaline (see fig. 6 and fig. 7). In

some way or other it must be a phenomenon related to the action of adrenaline. A plausible explanation might be that after the infusion is interrupted the concentration of l-adrenaline in the circulating blood steadily falls, and again passes through the range where the dilator action of l-adrenaline is more effective than its constrictor action. Another more probable mechanism is that the elimination of lactic acid from the skeletal muscle cells and the blood is a rather slow process. If this is the case, it is quite reasonable why the dilatation would reappear and continue for some time as the actual dilator agent is still present in the tissues and the blood.

The responses of blood vessels of the skeletal muscles to the adrenal medullary discharge at different frequencies almost parallel those of l-adrenaline infusion. From the work of EULER and others (see e. g. EULER and FOLKOW 1953) it is established that in the cat the proportion of noradrenaline in the medullary output may reach a considerable figure and consequently one would primarily expect a vasoconstriction and not a dilatation. The results in the present series are, however, quite in accordance with those on man of DE LARGY, GREENFIELD, MCCORRY and WHELAN (1950), who demonstrated that the dilator action of adrenaline was still pronounced in mixtures of the two catechols containing as much as 75 per cent noradrenaline. Further, if the total amount of catechols released from the adrenal medullae is moderate, the concentration of adrenaline might well reach the 'dilator threshold' long before the 'constrictor threshold' for noradrenaline is achieved. Thus the dilator action of adrenaline will dominate the picture in face of a rather low percentage of adrenaline, as it has a much lower threshold than the constrictor effects.

In fig. 9 the responses of the blood vessels of skeletal muscles to (*A*) stimulation of the constrictor nerves, (*B*) the adrenal medullary output, (*C*) l-adrenaline and (*D*) l-noradrenaline are summarized. It is natural that variations occurred in different animals. Therefore, this and the similar diagrams that follow, are only supposed to present the general trends in this study as regards the range of control of the different components. (It should be remembered that, in this series of experiments, the secretory nerves to the left adrenal gland only were stimulated and that the right adrenal was tied off or extirpated. The assumption was made that the total

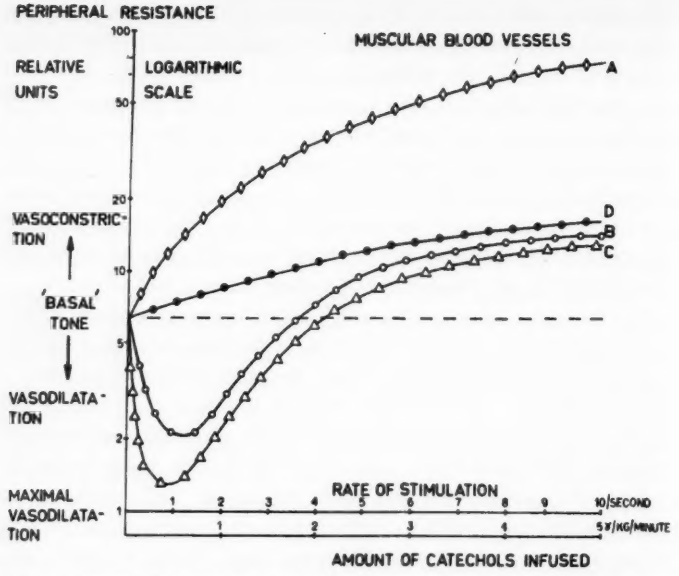

Fig. 9. Control of muscular blood vessels by sympathetic constrictor nerve fibres (curve *A*) and *both* adrenal medullae (curve *B*) — upper abscissa. Curves *C* and *D* show the effects of l-adrenaline (curve *C*) and l-noradrenaline (curve *D*) — lower abscissa. Note the marked 'basal' tone normally exhibited by sympathetically decentralized muscular blood vessels.

amount discharged from *both* adrenals at a given rate of splanchnic stimulation corresponds approximately to that liberated from *one* adrenal when activated at *double* that frequency. Such an approximation has to be done because of the technical difficulties in getting free access to the right adrenal gland. All the diagrams of this type, including fig. 9, are calculated to be valid for the responses to the medullary secretion from *both* the adrenals when activated at various rates of stimulation within the discharge range of sympathetic nerve fibres. That is to say, that the response of the blood vessels in the diagram to both the adrenal medullae activated at e. g. 4 impulses per second, was *actually* the response to the left gland only when activated at 8 impulses per second.)

It is evident that, at least in the cat, the sympathetic vasoconstrictor nerve fibres constitute the only factor of major importance

as regards eliciting constriction of the blood vessels in skeletal muscles. There is little or no co-operation between constrictor nerves and the adrenal medullae in this very respect. Even in cases when the medullary output consists more or less entirely of noradrenaline the constrictor action of noradrenaline from the adrenals cannot by far compete with the action of the constrictor nerve fibres. This will probably be true even in cases when the percentage of noradrenaline in the medullary output might be selectively increased (see e. g. the papers by EULER and FOLKOW 1953 and HILLARP and HÖKFELT 1953). The amounts of noradrenaline separately secreted from the adrenal medullae might reinforce the action of the constrictor nerves to some extent. However, the constrictor capacity of those amounts which the adrenals are capable to release during physiological conditions is very limited compared with the effects of the constrictor nerves when activated at similar rates. Further, any neurogenic vasoconstriction will limit the effectiveness of blood-borne substances simply because the reduced blood flow will decrease the amounts supplied.

Thus, as regards *vasoconstriction*, this vascular area is dominated by the constrictor nerve fibres to such an extent that influence of the adrenals might almost be neglected.

The dilator action of adrenaline is, on the other hand, a very potent factor capable of inducing a maximal dilatation even at quite small concentrations of adrenaline. It adds an important contribution to e. g. the dilatation which sympathetic cholinergic dilator fibres provoke in skeletal muscles. (For references see LINDGREN and UVNÄS 1954). However, there are, as mentioned previously, good reasons to conceive that this dilator action of adrenaline is secondary to the 'metabolic' glycogenolytic action of adrenaline, and not a true 'motor' effect directly on the vascular smooth muscles.

B. The blood vessels of the skin

In spite of the fact that more than a century has passed since BERNARD and BROWN-SEQUARD established the existence of vasoconstrictor nerves to the skin vessels, practically all our knowledge about the quantitative aspects of the action of these constrictor

fibres is of quite recent origin. GIRLING (1952) when stimulating the constrictor nerves to the rabbit's ear found a range of effective stimulation frequencies of one impulse every other second to 25 impulses per second. This question was also examined by CELANDER and FOLKOW (1953) who on the cat found extensive constrictor effects on the cutaneous blood vessels even at very slow rates of stimulation. The well-known facts that the circulation of the skin stands in a key position in the regulation of body temperature, and that exposure of the organism to cold is a potent stimulus for the adrenal medulla, raise the question to what extent the control of cutaneous blood flow is exerted on the one hand by the constrictor nerve fibres and on the other the constrictor hormones released from the adrenal medulla.

Method

Experiments were performed on twenty-five cats. The experimental approach was principally the same as that applied regarding the blood flow through skeletal muscles and has also been fully described in a previous paper (CELANDER and FOLKOW 1953). The vascular region investigated was the paw of the cat. To avoid pressor and depressor reflexes it was sympathetically decentralized. Reflex discharge from the adrenal medullae was eliminated as previously described and any interference by variations in blood pressure was counteracted by partial occlusions of the aorta. The big saphenal vein draining the main part of the paw was cannulated at the height of the ankle joint and other cutaneous veins at this height were ligated. The changes in cutaneous blood flow following stimulation of the regional sympathetic constrictor fibres were recorded as were the effects of the adrenal medullary discharge and a quantitative comparison was made. In addition the dose-response curves of l-adrenaline and l-noradrenaline on skin vessels were determined.

Results

The vascular bed of the skin of the cat's paw, acutely decentralized from the sympathetic nervous outflow, allows an almost maximal blood flow at an ordinary room temperature of about 20° C. This could easily be shown by giving acetylcholine 'close-arterially' which never increased the blood flow more than some 50 to 100 per

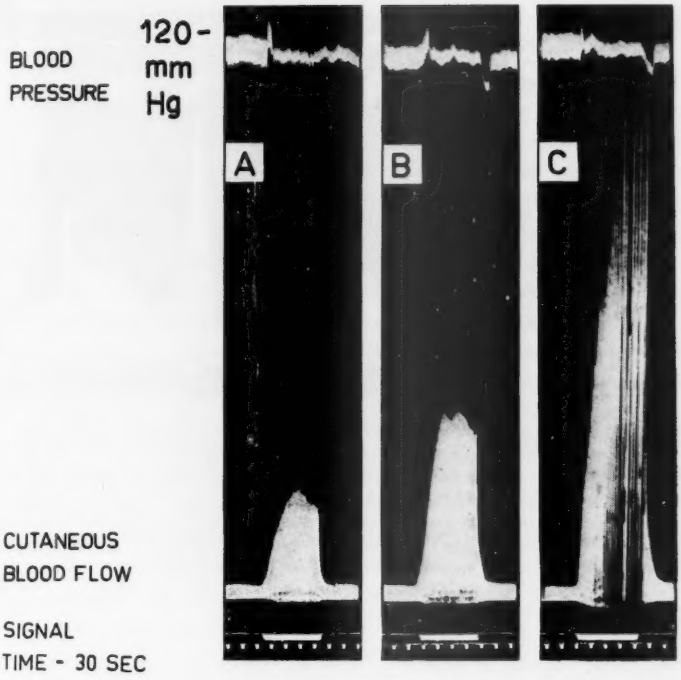

Fig. 10. Blood flow of a cutaneous vascular region. Effects of vasoconstrictor nerve stimulation. *A* — Stim. lower abdominal sympathetic trunk (30/min), *B* — Do. (1/sec), *C* — Do. (4/sec).

cent. This might be due to the abundance of arterio-venous anastomoses which probably dilate maximally when decentralized from the sympathetic nervous system. On the other hand, the 'nutritional' vascular pathways of the skin, which account for only a fraction of the total blood flow, might well still exhibit a fairly good 'basal' tone.

Stimulation of sympathetic vasoconstrictor fibres to the blood vessels of the skin

The exquisite sensitivity of skin vessels to constrictor nerve stimulation is apparent from fig. 10. Already at rates as low as *one impulse every fourth second*, a considerable rise in peripheral resistance

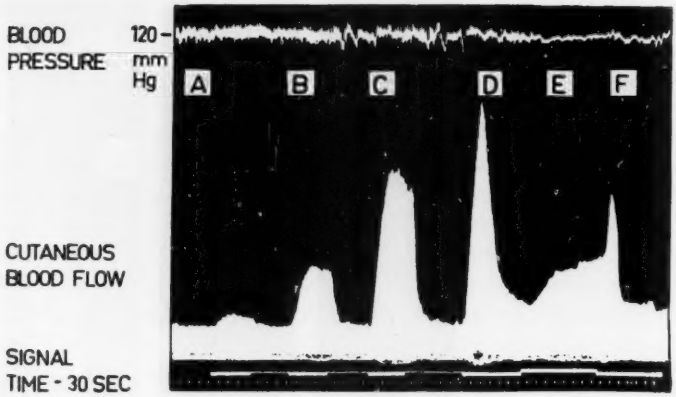

Fig. 11. Effects of the adrenal medullary discharge on cutaneous blood flow. *A* — Stim. left splanchnic nerves (2/sec), *B* —Do. (4/sec), *C*— Do. (8/sec), *D* — Do. (16/sec) — note the rapid *decline* of vasoconstriction to almost 'basal' level in spite of continued stim. of left splanchnic nerves. *E* — Do. — direct shift to 6/sec — note the gradual *return* of vasoconstriction when the rate of stim. was lowered into the 'physiological' range of sympathetic nerve fibre discharge. *F* — Do. — direct shift to 14/sec — note initial slight increase of vasoconstriction which, however, rapidly fails.

was mostly obtained. See e. g. *A* in fig. 13 where the rate of blood flow was reduced to about 20 per cent of 'basal' level indicating a fivefold increase of peripheral resistance. Almost maximum constrictor effects — which in many animals implied that the blood flow through the region practically ceased, and indicated roughly a hundredfold increase of resistance to blood flow — were obtained at stimulation rates not exceeding 10 impulses per second.

The adrenal medullary discharge on the blood vessels of the skin

The left splanchnic nerves were maximally stimulated at various frequencies and the effects of the discharged medullary hormones on the blood flow through the skin vessels, decentralized regarding the constrictor nerve pathways, were quantitatively recorded at a perfusion pressure that was kept constant. A typical experiment of this kind is shown in fig. 11.

The reduction of cutaneous blood flow that follows upon left

splanchnic nerve stimulation was quite moderate (at least when compared with the extensive neurogenic vasoconstrictions obtained at low rates). It was sustained for the period of adrenal medullary excitation at frequencies up to about 8 per second. However, fig. 11 demonstrates an interesting observation with a bearing on other aspects of the present study. At *D* in fig. 11 the splanchnic nerves to the left adrenal were stimulated at a rate of 16 impulses per second. But in spite of a continued stimulation there was a rather rapid decline of the vascular response almost down to initial level. This could possibly not be due to some kind of fatique of the muscle cells, because there was no failure of the skin vessels in keeping a much more intense vasoconstriction sustained in response to vasomotor nerve stimulation (fig. 10) or intravenous administration of l-adrenaline (fig. 12). In case of l-noradrenaline infusion there was sometimes a moderate decline of initial response (fig. 12) but never down to 'basal' values. The most suggestive explanation for the progressive decline of vasoconstriction following upon left splanchnic nerve stimulation at higher rates is of course that the catechol stores in the adrenal medullary cells must be more or less emptied. However, if the rate of left splanchnic nerve stimulation was — without any delay — turned down to 6 impulses per second (*E*) there was a gradual return of cutaneous vasoconstriction. Obviously, there must still have been catechols available in the adrenal medulla. The reason for the decline might have been a failure of the praeganglionic sympathetic nerve fibres to the adrenal medullary cells in the splanchnics to transmit nerve impulses at a rate of 16 impulses per second. According to what is known about the diameter and electrophysiological characteristics of those nerve fibres this can hardly be the case. As far as I can see the revelant mechanism must be a failure of the cholinergic synaptic transmission of the nerve impulses between praeganglionic nerve fibres and the adrenal medullary cells. If, due to exhaustion of the release mechanism, the amount of acetylcholine per single shock stimulus is successively reduced it will soon reach subthreshold amounts. Due to the momentary destruction of the transmitter by the cholinesterase an additive action of subsequently released subthreshold quanta is made impossible. The excitation of the gland cells which obey the all-or-none law will then fail. The present

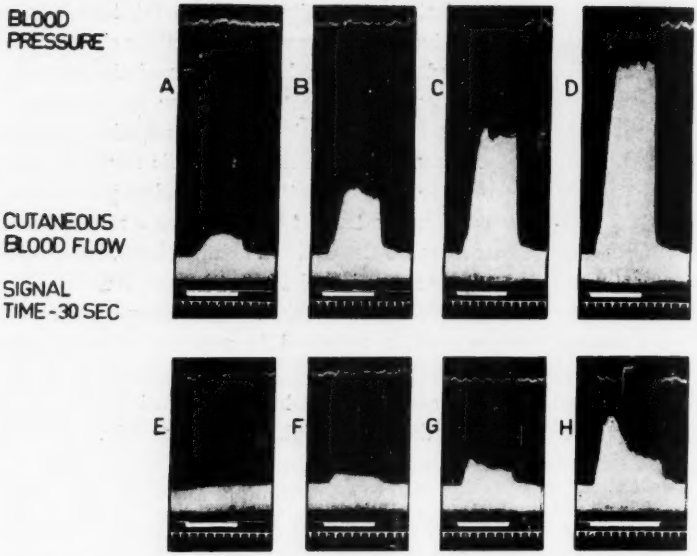

Fig. 12. Effects of l-adrenaline and l-noradrenaline on cutaneous blood flow. 'Perfusing' blood pressure 120 mm Hg. *A* — I. v. infusion l-adrenaline (0.3 μg/kg/min), *B* — Do. (0.7 μg/kg/min), *C* — Do. (1.4 μg/kg/min), *D* — Do. (3 μg/kg/min), *E* — I. v. infusion l-noradrenaline (0.3 μg/kg/min), *F* — Do. (0.7 μg/kg/min), *G* — Do. (1.4 μg/kg/min), *H* — Do. (3 μg/kg/min). Compare *A*—*E*, *B*—*F*, *C*—*G*, *D*—*H*, where equal amounts of the two catechols were administered.

findings are in accordance with the rapid failure of acetylcholine release in a sympathetic ganglion upon higher rates of praeganglionic stimulation reported by PERRY (1953) and the decline of action potentials in sympathetic postganglionic nerve fibres upon praeganglionic stimulation at rates higher than about 20 per second (see BRONK 1939). A contributary factor might be the depressive action of catechols on synaptic transmission in the sympathetic nervous system which was postulated by MARRAZZI (1939). The present observation on the adrenal medullary secretion at different rates of praeganglionic stimulation adds a piece of suggestive evidence as to the upper limit of impulse frequence of the sympathetic nervous system.

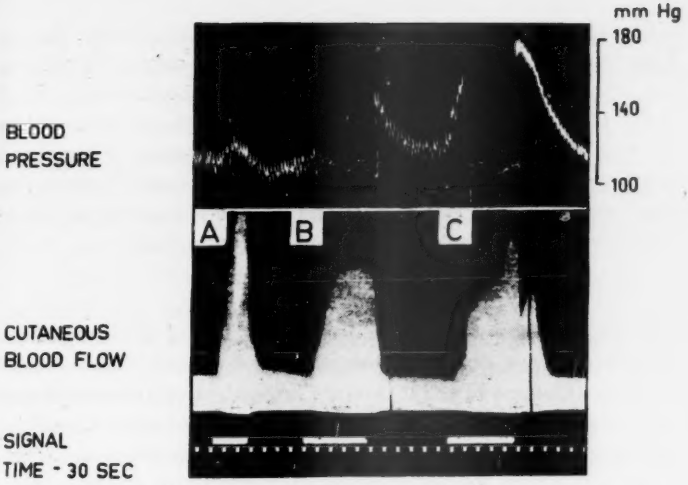

Fig. 13. Cutaneous blood flow. *A* — Stim. lower abdominal sympathetic trunk (*15/min*), *B* — Stim. left splanchnic nerves (8/sec), *C* — I. v. infusion of l-adrenaline (50 %) and l-noradrenaline (50 %) — in total 1.5 μg/kg/min. Note the extensive response to the very low rate of constrictor nerve stimulation (*A*), and that a much higher rate of stimulation to the left adrenal is necessary to obtain a similar vasoconstriction (B).

Intravenous infusion of l-adrenaline

Doses up to 0.1 μg/kg/min did not affect skin vessels. An increase to 0.3 μg/kg/min quite regularly reduced blood flow to about fifty per cent (*A* in fig. 12). Still larger amounts produced a further increase of peripheral resistance (*B*, *C*, *D* in fig. 12). At *D* 3 μg/kg/min caused an eightfold increase of peripheral resistance. The vasoconstriction was maintained throughout the period of infusion even when lasting more than ten minutes. In no case did the skin vessels show a 'secondary dilatation' beyond the 'basal' level after the infusion of l-adrenaline was interrupted, as was regularly the case with the vessels of the muscles.

Intravenous infusion of l-noradrenaline

Fig. 12 demonstrates that l-noradrenaline had a definitely weaker constrictor action upon cutaneous vessels than had l-adrenaline which was a consistant finding in all experiments.

Doses less than 0.3 μg/kg/min seldom affected skin vessels. Larger doses elicited rises of peripheral resistance but only to about one half or one third of an equal concentration of l-adrenaline. At *F* in fig. 12 0.7 μg/kg/min increased peripheral resistance about thirty per cent. At *G* 1.5 μg/kg/min gave a twofold increase of peripheral resistance and at *H* 3 μg/kg/min a fourfold increase. However, in these concentrations (see e. g. *G* and *H*) the constrictor action of l-noradrenaline was not sustained but declined rapidly.

Comments

The wide range of skin vessel control by way of the sympathetic constrictor nerve fibres and the readiness by which quantitatively important changes of blood flow are brought about, even at a very slow rate of stimulation, are obvious. Almost maximal constrictor responses were obtained in most animals at frequencies that seem to be well within the physiological discharge range of sympathetic nerve fibres.

In the recent study by GIRLING (1952) frequencies below one impulse every other second proved ineffective in the rabbit's ear. This is not the experience of the present author as regards the cat's paw as it was quite a common finding in this series, adopting a similar method as GIRLING, that frequencies below one impulse every other second could cause a great change in the vascular resistance (see for instance *A* in fig. 13). There is, however, some evidence that stimulation of the corresponding cervical sympathetic trunk will not include all vasoconstrictor fibres to the rabbit's ear (see FELDBERG 1926) which may, at least in part, account for the ineffectiveness of lower frequencies as reported by GIRLING. Further, the differences in experimental condition including type and level of anaesthesia, method of recording blood flow and the fact that our studies were not undertaken on the same species, might be responsible for this divergence.

By intravenous infusion of l-adrenaline and l-noradrenaline or by stimulation of the nerves to the adrenal medulla it was possible to constrict the skin vessels to a rather marked degree. A study of the literature on this topic seems to indicate that noradrenaline has a stronger pressor capacity and might thus have a more marked constrictor effect on the blood vessels than has adrenaline. The

arterial blood pressure, however, cannot be considered primarily as a reliable guide regarding the vascular actions of a drug in a given area, since it represents the balance of its dilator and constrictor actions in the body. Besides, the changes in blood pressure might be related to changes in cardiac output induced by the drug. To get closer information about the true constrictor or dilator action on different vascular areas, quantitative measurements of their effects on peripheral resistance to blood flow in these regions must be performed. The skin vessels do not seem to be surrounded by any tissue that could be expected to modify the responses of the vascular smooth muscle cells to any larger extent (as might be the case with the vessels in the skeletal muscles). This vascular region, therefore, is of a special interest for a quantitative study of the direct constrictor actions of adrenaline and noradrenaline on blood vessels. In the present series, the two catechols were infused in amounts up to what can be considered to be the maximum capacity of the adrenals (see Chapter II, p. 27). Interference from nervous, hydrostatic and humoral factors were on the whole eliminated. In no animal l-noradrenaline had a stronger constrictor action on skin vessels than l-adrenaline. The ratio for 'equiconstrictor' doses of l-noradrenaline and l-adrenaline (N/A) was found to vary from 2/1 to 5/1 in different animals. In other words, in order to obtain a certain degree of vasoconstriction l-noradrenaline had to be infused in amounts 2 to 5 times that of l-adrenaline.

The results in the present series are summarized in fig. 14 which shows the general trends of skin vessel responses to (*A*) stimulation of the vasoconstrictor nerve fibres, (*B*) the adrenal medullary output (this curve is calculated to represent the responses to *both* adrenals — see p. 47), (*C*) l-adrenaline and (*D*) l-noradrenaline. At a first glance the responses to the adrenal medullary hormones from a quantitative point of view are quite impressive. There are, however, some facts that should be kept in mind.

(i) Skin vessels that are acutely decentralized from the sympathetic nervous system are almost dilated to maximum. That means that the measurements of the constrictor action of the two catechols have all been made on the steepest part of the 'dose-response' curve.

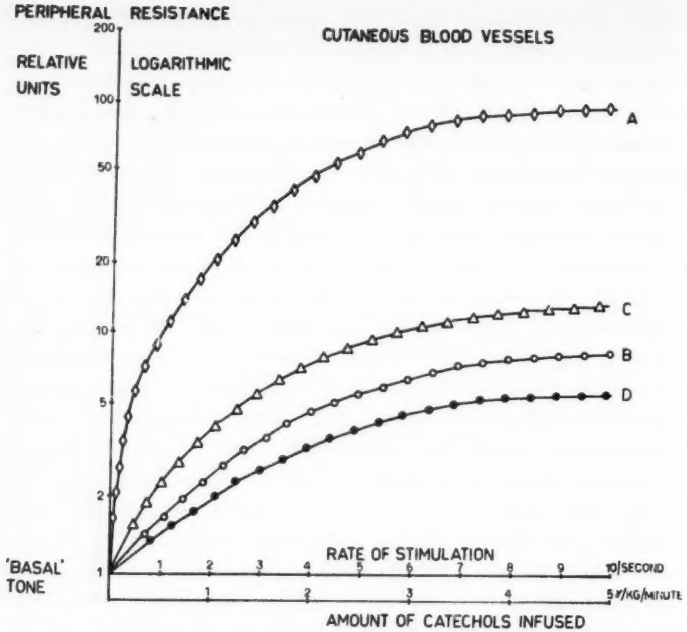

Fig. 14. Control of cutaneous blood vessels by sympathetic constrictor nerve fibres (curve *A*) and *both* the adrenal medullae (curve *B*) — upper abscissa. Curves *C* and *D* show the effects of l-adrenaline (curve *C*) and l-noradrenaline (curve *D*) — lower abscissa.

(ii) Although the maximum total output of catechols from the two adrenal glands was found to be roughly 5 μg/kg/min when all the cells were maximally activated at the top of the physiological discharge range, the amounts released at strong reflex activation of the adrenal medulla probably do not exceed 1—3 μg/kg/min (see e. g. Rapela and Houssay, 1952 and Euler and Folkow, 1953). The present experiments, therefore, put the adrenal medullae in a favourable position regarding their quantitative importance for the regulation of cutaneous blood flow, and the effects should be looked upon as the theoretically most optimal and maximal ones.

(iii) The dilated skin vessels admitted a blood flow that was considerably larger than would be the case if the vasomotor nerves were

left intact. Consequently, a relatively larger proportion of the infused substances will reach this area. In a region with a normal or marked vasomotor tone, the blood flow, and as a consequence the amounts and effectiveness of blood-borne substances supplied, will be proportionally reduced.

(iv) Any more marked reflex activation of the adrenal medullae is part of an 'emergency reaction' including an increase of activity in most components of the sympathetic nervous system, and probably then at a relatively uniform discharge rate. In case of skin vessels the medullary discharge has indeed an overwhelming competitor in the vasoconstrictor innervation which is capable of inducing more than a hundredfold increase of resistance to blood flow. These two components will co-operate, but the responses to the weaker — which is the adrenal medulla — will in most cases be quite overshadowed by those induced by the constrictor fibres. It can be claimed that, as a rough estimate, the constriction of skin vessels in response to stimulation of the constrictor nerves were generally some 10 to 20 times bigger than those brought about by the adrenal medullae when stimulated at similar rates.

C. The blood vessels of the kidney

In most mammals renal blood flow during resting conditions accounts for some 20—25 per cent of total cardiac output. Thus, the resistance to blood flow exerted by the renal vascular bed is very low, when considering the small fraction of total body weight which the kidneys constitute. Any change of the resistance to blood flow in the kidneys might therefore have pronounced effects on arterial blood pressure and consequently be a factor of prime importance in circulatory homeostasis under certain conditions. However, there is a considerable amount of experimental and clinical evidence (see e. g. Smith 1951) indicating that this vascular region to a rather slight extent participates in various pressor and depressor reflexes, at least when these are of moderate degree, and mostly in the intact individual it has a rather remarkable ability to keep its blood flow relatively constant in spite of changes in arterial blood

pressure and vascular adjustments elsewhere. In 1947, TRUETA, BARCLAY, DANIEL, FRANKLIN and PRICHARD introduced their concept about the 'two potential circulations' through the kidney — one associated with the cortical glomerulis and the other with the 'juxtamedullary' — and that during various 'extreme conditions' a redistribution of blood flow within the kidney between these two pathways might occur.

Studies on renal haemodynamics originating from the work of TRUETA et al. (1947) have appeared in a large number during recent years and demonstrate great contradictions as to the existence and functional consequencies of the postulated 'Trueta shunt'. Some of them (see e. g. DANIEL, PEABODY and PRICHARD 1951) confirm the dual nature of the intrarenal circulation but stress that both pathways are to be looked upon as glomerular (DANIEL, PEABODY and PRICHARD 1952). Others (see e. g. BLOCK, WAKIM and MANN 1952 a, HOUCK 1951, a and b, and SCHER 1951) doubt the existence of the renal vascular shunt mechanism, while still others (e. g. DEL POZO, HERNANDEZ-PEON and GUZMAN 1952) look upon the deviation of cortical blood flow to deeper regions in the kidney as a transitory phenomenon. See also BERGSTRAND (1952).

In the study of renal haemodynamics the possibility of important intrarenal redistributions of blood flow according to the 'Trueta-mechanism', and changes of renal function, in spite of a relatively stable total blood flow through the kidney, must be kept in mind.

The aim of the present investigation was to bring forward an estimate of the relative effectiveness of the vasomotor nerves and the adrenal medullary hormones in the regulation of the *total* amount of blood flowing through the kidneys, thus taking into consideration the *net* peripheral resistance of the renal vessels but without any efforts to reveal any possible simultaneous intrarenal redistributions of blood flow.

Method

Experiments were performed on eleven cats. Because of the magnitude of renal venous outflow and the inability of the 'drop-recorder' to cope with a drop rate exceeding about five drops per

second small cats of 1.5—2 kg of body weight had to be used in this series.

Under magnification the sympathetic constrictor fibres to the left kidney joining the renal vessels were carefully isolated, tied together by a silk thread and centrally cut. The peripheral part of this bundle was placed on an Ag-AgCl-electrode. The indifferent electrode was fastened to the abdominal wall. A cannula was inserted into the left renal vein and by way of a polyethylene tube running through a small incision in the right part of the abdominal wall the venous outflow was directed to the 'drop-recorder'. The sole vascular pathways between the left kidney and the rest of the animal's body were the main renal artery and vein. Other vessels were ligated. The 'perfusing' blood pressure was recorded in the right femoral artery and was kept constant at about 120 mm Hg by adjusting a screw-clamp on the aorta just proximal to the renal arteries. Reflex adrenal medullary discharge was eliminated as previously described. Thus the changes in renal blood flow induced by constrictor nerve stimulation, by the adrenal medullary output at different frequencies of left splanchnic stimulation, as well as, by the intravenous infusions of l-adrenaline and l-noradrenaline could be separately investigated.

Results

Stimulation of sympathetic vasoconstrictor fibres to the blood vessels of the kidney

The results obtained in this series were highly variable and inconsistent. In some animals the vasoconstriction brought about by stimulation, even at rates as high as 10 to 20 per second was quite moderate and in others the constrictions elicited rapidly declined. In some animals, however, it was possible to induce a rather extensive vasoconstriction at stimulation rates that were considered to be 'physiological' — that is not exceeding 10 per second — and the constrictions were maintained at least for some minutes. It is not very surprising why in some animals the stimulation of the sympathetic nerve fibres to the kidney proved to be relatively ineffective compared with other experiments. These vasoconstrictor nerves consist of tiny fibres which are difficult to locate and isolate and very easy to damage — for instance by

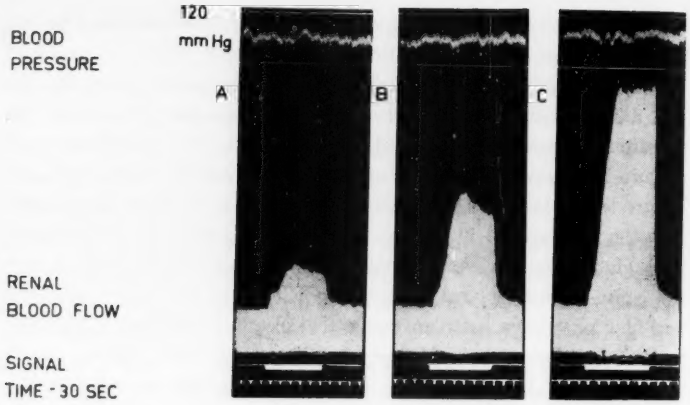

Fig. 15. Renal blood flow. Effects of vasoconstrictor nerve stimulation. *A* — Stim. renal nerve fibres (3/sec), *B* — Do. (6/sec), *C* — Do. (12/sec).

traction — during the preparation. Therefore those animals which showed marked increases of resistance to blood flow to sympathetic stimulation, and in which the magnitude of the responses varied in proportion to the rates of stimulation applied, were considered to be truly representative for this series of experiments.

Fig. 15 shows the result in one of those experiments that were considered representative regarding the effects of the renal vasomotor nerves. At *A* the frequency of stimulation was 3 per second. The rate of blood flow decreased to some 50 per cent of the initial level. In most animals attempts to evoke a definite vasoconstriction at rates lower than 2 to 3 per second proved unsuccessfull. At *B* the stimulation of renal constrictor fibres was repeated at a frequency of 6 per second. The blood flow now decreased to some 20 per cent as compared with initial level. An almost maximal vasoconstriction was reached at 12 impulses per second (*C*) indicating a sixfold decrease of blood flow. Any further increase of the rate of stimulation did not increase the response very much. It must be realized that even in these apparently successful experiments it is possible that only a fraction of the renal vasomotor nerves were stimulated, so that, in fact, the range of vasomotor nerve control of the renal circulation is still wider.

The adrenal medullary discharge on the blood vessels of the kidney

The activation of the left adrenal medulla was achieved, as previously, through stimulation at various frequencies of the left splanchnic nerves. The responses of the renal vessels, acutely decentralized from the sympathetic nervous system, were quantitatively recorded at a constant perfusing pressure of 120 mm Hg. From a quantitative point of view the effects on the net renal blood flow were not very pronounced. A definite vasoconstriction was not achieved until the rate of splanchnic stimulation was increased up to a rather high level. The maximal response revealed that the resistance to blood flow was about doubled. It is difficult to be quite sure that the denervation of renal vessels described under 'Method' was absolutely complete. If not, splanchnic stimulation would activate the remaining sympathetic constrictor fibres as well as the fibres to the left adrenal medulla, and thus, as an unwanted artifact, reinforce the action of the medullary hormones. To be certain, the renal pedicle in some animals was infiltrated by a local anaesthetic (procaine). This procedure, however, did not, to any larger extent, alter the responses of the renal vessels to splanchnic stimulation and, it was concluded that the denervation of the kidney was, from a functional point of view, complete.

Intravenous infusion of l-adrenaline and l-noradrenaline

When given at a small dosage both substances proved ineffective in reducing renal blood flow as measured by recording the venous outflow. Even in amounts up to 1 μg/kg/min — which, regarding a discharge from the suprarenal medulla, is a relatively large amount — there was no consistent decrease of blood flow at all in most of the experiments. When l-adrenaline was infused at a rate of about 2 μg/kg/min the renal response was a slight decrease of blood flow with an increased resistance of some 25 per cent. In order to decrease the blood flow to the kidney to half the 'control' value, the dose of l-adrenaline and l-noradrenaline necessary, was found to average 5 to 8 μg/kg/min — that is, amounts probably much bigger than that released during any type of strong reflex activation of the adrenals in the intact organism. See fig. 16.

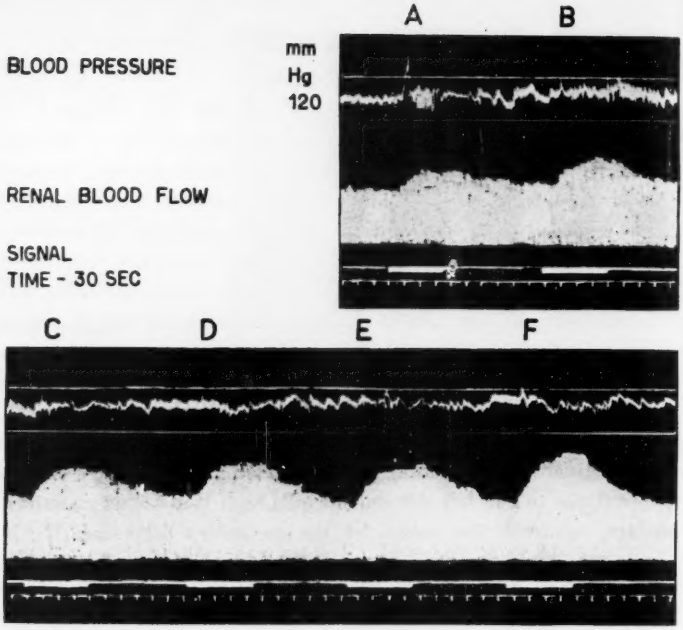

Fig. 16. Effects of l-adrenaline and l-noradrenaline on renal blood flow. *A* — I. v. infusion l-noradrenaline (2.2 μg/kg/min), *B* — Do. l-adrenaline (2.2 μg/kg/min), *C* — Do. l-noradrenaline (4 μg/kg/min), *D* — Do. l-adrenaline (4 μg/kg/min), *E* — Do. l-noradrenaline (7.2 μg/kg/min), *F* — Do. l-adrenaline (7.2 μg/kg/min).

In all animals l-noradrenaline had a definitely weaker constrictor action on renal vessels than had l-adrenaline. The ratio for 'equi-constrictor' doses of l-noradrenaline and l-adrenaline varied in different animals but generally, l-noradrenaline had to be infused in amounts about twice as large as those of l-adrenaline in order to provoke the same degree of vasoconstriction.

Comments

During recent years, numerous investigations have been published on renal haemodynamics, both in man and in different species of laboratory animals. The methods applied constitute in some instances a determination of the actual renal blood flow, for instance

by cannulating the renal vein (see e. g. the papers by SELKURT 1946 and later). Of the indirect methods the most frequently used is based on PAH clearance. Working on dogs in haemorrhagic shock SELKURT (1946) was able to demonstrate that these two types of renal blood flow determinations did not parallel under all conditions. During the periods of hypotension following bleeding, clearances went to zero in spite of the fact that considerable amounts of blood were still flowing through the kidney. Clearance tests might give information about 'effective plasma flow' but, to achieve safe knowledge about changes in resistance to blood flow a direct method should be applied.

When the adrenal medullary hormones are released or infused, the renal blood flow (as is also the case with other intact vascular areas) might be affected in at least three different ways.

(i) By the direct effect of these substances on the smooth muscle cells of the renal vessels.

(ii) By changes in arterial blood pressure.

(iii) Any change of blood pressure is counteracted in the intact organism by compensatory changes of the tonic discharge in sympathetic constrictor fibres by way of the baroreceptor and chemoreceptor influences upon the activity of the vasomotor centre.

It has been argued (see e. g. SMITH 1951) that there is no tonic action of the sympathetic vasomotor fibres to the renal blood vessels during resting conditions, and a more or less open question is the degree of participation of this vascular region in circulatory homeostasis (e. g. upon pressor and depressor reflexes *via* the baroreceptor and chemoreceptor regions, and in various states of emergency). See for instance the papers of BEZNÁK and LILJESTRAND (1952) and PAGE and MCCUBBIN (1953). In the present series the mechanisms (ii) and (iii) were ruled out and any change of renal blood flow in response to the medullary hormones could be attributed to the direct effect on renal vessels.

The circulation through the kidney and the renal function of the dog during stimulation of the renal nerves have been studied, for instance, by KOTTKE, KUBICEK and VISSCHER (1945), HOUCK (1951 b) and BLOCK, WAKIM and MANN (1952, a and b). In the experiments

by KOTTKE et al. (1945) renal blood flow was measured by a type of 'thermostromuhr' applied to the renal vein. In regard to their method of stimulation the objection may be raised that they did not transect the renal nerves centrally. Thus, the responses of the renal vessels might, in part, be attributed to a reflex activation of the vasomotor centre and the adrenal medullae following a possible simultaneous stimulation of afferent pain fibres. However, their results are important and demonstrate that the renal blood flow could be drastically reduced by low frequency, low voltage stimulation of renal nerves. Stimulation at higher rates, however, led to an early decline of the blood vessel response. In some dogs renal blood flow was decreased to about 10 per cent of control rate. To produce a prolonged reduction of renal blood flow the frequencies used must not exceed 5 impulses per second — otherwise fatigue readily occurred. (See also KUBICEK, KOTTKE, LAKER and VISSCHER 1953.)

The responses of renal vessels to the administration of l-adrenaline and l-noradrenaline have been studied both by direct methods (see e. g. KREIENBERG 1950, SCHER 1951 and PAGE and MCCUBBIN 1953) and indirectly by clearance tests (see e. g. WERKÖ, BUCHT, JOSEPHSON and EK 1951, PICKFORD and WATT 1951, BERNE 1952 and KAPLAN, FOMON and RAPOPORT 1952). For the literature on this matter see e. g. KAPLAN et al. (1952). Some studies (e. g. TRUETA et al. 1947, and HOUCK 1951 a) appear to be of little or no relevance regarding information concerning the effects on renal blood flow by the adrenal glands during physiological conditions, because the amounts administered are far beyond what can be considered as 'physiological'. The results obtained by direct and indirect methods are somewhat controversial but the general evidence appears to indicate that clearance tests may be moderately influenced by amounts up to some 2 μg/kg/min (see e. g. WERKÖ et al. 1951) but, that rather high amounts have to be given in order to obtain a definite vasoconstriction as recorded by a more direct method (see e. g. KREIENBERG 1950 and PAGE and MCCUBBIN 1953).

Different opinions are reported as to the relative effectiveness of l-adrenaline and l-noradrenaline in constricting renal vessels and influencing renal function. The results of KAPLAN et al. (1952), in the dog, did not support the view that the potency of l-noradrenaline to reduce renal blood flow is greater than that of l-adrenaline

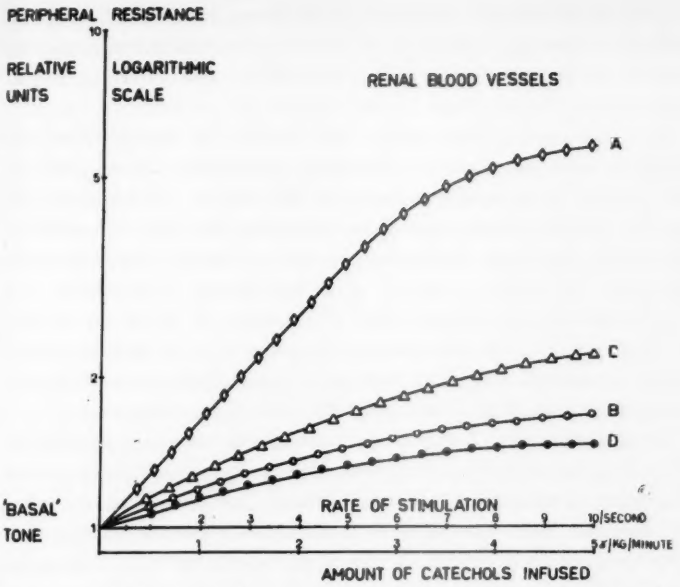

Fig. 17. Control of renal blood vessels by sympathetic constrictor nerve fibres (curve *A*) and *both* adrenal medullæ (curve *B*) — upper abscissa. Curves *C* and *D* show the effects of l-adrenaline (curve *C*) and l-noradrenaline (curve *D*) — lower abscissa.

when giving comparatively small amounts of these compounds. Their estimates were based on clearance tests. This is in keeping with the opinion of BURN and HUTCHEON (1949) who concluded that adrenaline reduced renal blood flow more effectively than noradrenaline. It is, however, contrary to the view put forward by WERKÖ et al. (1951), based on renal extraction of PAH, that in normal human subjects noradrenaline had a more pronounced effect than adrenaline in this respect.

In the present series on cats, noradrenaline had a definitely weaker constrictor action on renal vessels than had adrenaline. Any interference by hydrostatic and nervous factors could be eliminated here.

The general trends in this series are diagramatically put together in fig. 17 which shows the responses of renal vessels to (*A*) stimula-

tion of sympathetic constrictor nerve fibres, (*B*) the adrenal medullary output (this curve is calculated to represent the responses to *both* adrenals — see p. 47), (*C*) l-adrenaline and (*D*) l-noradrenaline.

It is important to stress that a determination of the rate of venous outflow from the kidney only gives information about the net changes in peripheral resistance of this region. Considering the highly complex architecture of the renal vascular bed with afferent arterioles, glomerular capillaries, efferent arterioles, peritubular capillaries, venules and a variety of arterio-venous anastomoses, the exact number and physiological significance of which are poorly understood, the possibility always remains that, in spite of a relatively unchanged net blood flow, the infusion of these catechols may bring about important intrarenal circulatory adjustments.

It is seen in fig. 17 that the renal vascular responses to stimulation of the renal nerves at a given rate are approximately some ten times bigger than those following activation of the adrenal medullae at a similar rate. Furthermore, it should be remembered that it is especially difficult to pick up and stimulate *all* the renal vasomotor nerves. That means that the neurogenic effects in the intact animal most probably will be still more dominant.

D. The spleen

The role of the spleen in circulatory homeostasis has been a matter of dispute since, in 1723, STUKELEY (cit. MCDOWALL 1938) put forward the view that this organ had an important function as a blood reservoir. Much of the experimental evidence to support this view was presented by BARCROFT and co-workers. For the literature on this matter see e. g. BARCROFT (1926) and MCDOWALL (1938). According to BARCROFT (see BARCROFT et al. 1927) the regulation of splenic volume is primarily maintained through the action of the sympathetic constrictor fibres. Others (see e. g. HOLTZ and SCHÜMANN 1949) make the adrenal medulla more or less responsible for this task. According to these workers the participation of the spleen in the circulatory adjustments following occlusion of the carotid arteries, in the cat, is not very evident and mainly attributed

to the adrenal medullary discharge. This is contrary to the results of DRIVER and VOGT (1950) who, in the same animal, found that closure of the carotid arteries caused a contraction of the spleen, reflexly mediated by the splenic nerves, whether the adrenals were left intact or not.

The present study was performed to bring forward an estimate about the range of control on spleen size exerted by the splenic constrictor nerves as compared with the effects of the adrenal medullary hormones.

Method

Experiments were performed on eighteen cats. The intestines and the great omentum were extirpated in order to get free access to the spleen, the adrenals and the abdominal aorta. All vessels between the spleen and the stomach were ligated and the sole vascular pathways left intact were the main splenic artery and vein. The sympathetic nerve fibres, joining the splenic vessels, were cautiously isolated under magnification, tied together by a silk thread and centrally cut. The splenic artery and vein were freed from connective tissue sheaths and these vessels thus formed the only intact connections between the spleen and the rest of the animals body. The arterial 'perfusing' pressure, as recorded from one of the femoral arteries, was kept constant by the screw clamp on the aorta just below its entrance into the abdomen. The left splanchnic nerves were isolated and centrally cut. The right adrenal was extirpated and the left one could be excited by electrical stimulation of the peripheral ends of the left splanchnic nerves. The constrictor nerve bundle to the spleen was placed on an Ag-AgCl-electrode and could be maximally stimulated at various frequencies. To record the contractions of the spleen a modification of the plethysmograph, known as the 'oncometer', is the most commonly applied method. The hazards of this method are mainly the risk of leakage and the difficulties in avoiding mechanical interference with the splenic blood vessels. Therefore, in the present study spleen size was recorded by a photographic method. In the cat the shape of the spleen is variable in different animals. However, its surface facing the abdominal wall is quite smooth and flat and can practically always be placed in a horizontal plane without inflicting any hind-

rance to the circulation through the organ. After placing the stimulation electrode in position nothing had to be touched between the periods of stimulation. By taking a series of photographs, the 'control' size as well as the contractions elicited by constrictor nerve stimulation or, by the adrenal medullary hormones, could be conveniently recorded. These negatives were put into an enlarger and projected on sheets of ordinary paper. The border of the spleen was drawn and a measure for the surface area could be calculated by planimetry or simply by weighing the part of the paper occupied by the splenic projection. The magnitude of splenic contraction was expressed as the percental reduction of splenic surface area. However, as the arrangement of the smooth muscle cells in the capsule and trabecular framework of the spleen is quite irregular in all planes, the decline in splenic volume will probably approximately parallel that of the surface area.

Results

Stimulation of the splenic nerves

The spleen could be made to contract drastically by low frequency stimulation of the splenic nerves. The effects were marked at rates as low as *1 impulse every other second* and already at a frequency of 2 impulses per second the splenic contraction was almost completed in many experiments and any further increase of stimulation rate added comparatively small changes. Fig. 18 gives the record of an experiment which is considered to be representative for this group. A frequency of 1 impulse every other second reduced the spleen surface to about 85 per cent, 1 per second to 70 per cent, 2 per second to 55 per cent of control value. Still higher rates did not increase the splenic contraction to any significant degree.

The adrenal medullary discharge on the spleen

The left adrenal gland was activated by way of the left splanchnic nerves which were maximally stimulated at various rates. Fig. 19 shows the splenic responses obtained in the same animal that was referred to above (see fig. 18).

As seen from fig. 19 it was possible to provoke a marked contraction of the sympathetically decentralized spleen by stimulation of the

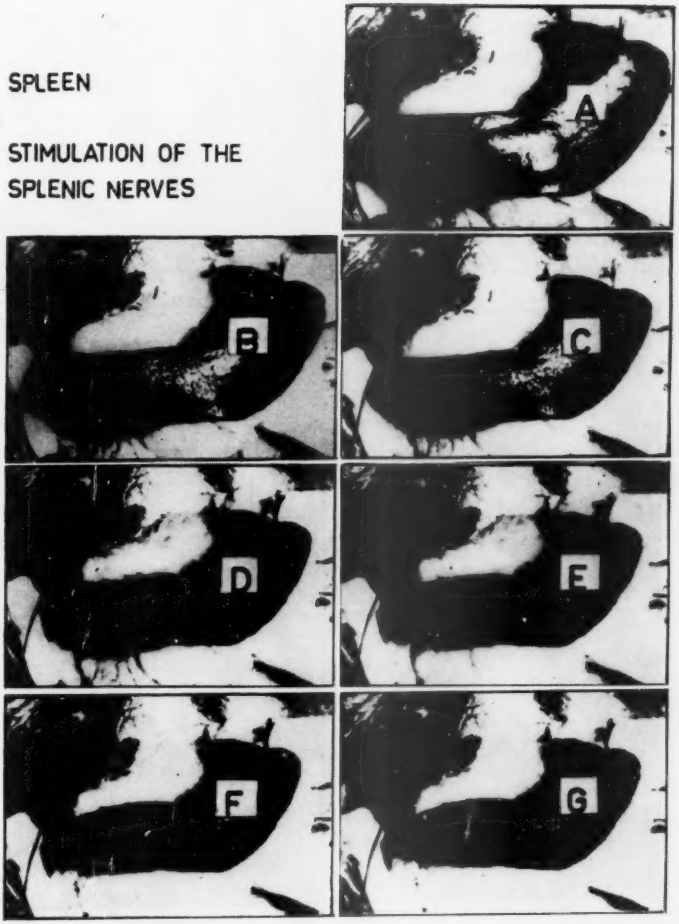

Fig. 18. *A* — Control, *B* — Stim. splenic nerve fibres (30/min), *C* — Do. (1/sec), *D* — Do. (2/sec), *E* — Do. (4/sec), *F* — Do. (8/sec), *G* — Do. (16/sec).

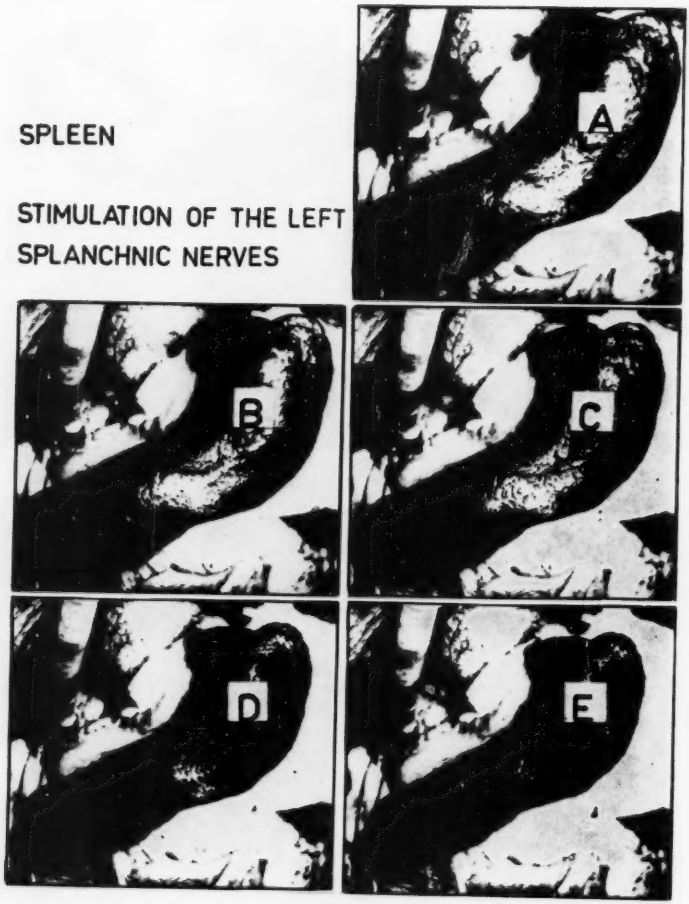

Fig. 19. Same animal as referred to in fig. 18. *A* — Control, *B* — Stim. of left splanchnic nerves (2/sec), *C* — Do. (4/sec), *D* — Do. (8/sec), *E* — Do. (16/sec).

left splanchnic nerves. These contractions must be attributed to the release of medullary hormones. However, at a low frequency stimulation of the splanchnic nerves the splenic response was moderate and the maximum response, induced by the adrenal medullary output, quite regularly did not reach the same high level as did the responses to constrictor nerve stimulation. The possible reasons for this divergency are discussed in the following 'Comments'. Further, it is seen from figs. 18 and 19 that in the lower region of the physiological discharge range of sympathetic fibres the predominance of the direct sympathetic innervation towards the adrenal medullae is marked. The splenic nerve fibres effect drastic constrictions at a surprisingly low rate of stimulation. To achieve the same response on the spleen the adrenal medullae have to be activated at rates some 5 to 8 times higher than the rates necessary in case of the direct innervation.

Intravenous infusion of l-adrenaline and l-noradrenaline

Both substances are effective in causing a splenic contraction. A definite contraction occurred when l-adrenaline was infused at a rate of 0.2 μg/kg/min and a maximum was reached at some 2 μg/kg/min. Intravenous infusion of l-noradrenaline proved less effective in all animals and in general the ratio for 'equiconstrictor' doses of l-noradrenaline and l-adrenaline (N/A) exceeded 2/1. See fig. 20 which shows the dose-response curves of these catechols on the sympathetically decentralized spleen at a constant 'perfusing' pressure.

Comments

The most outstanding feature in the present series is the exquisite sensitivity of the smooth muscle cells of the spleen to the excitation of its sympathetic innervation. At rates as low as *one impulse every other second* the splenic shrinkage was marked and in many animals 80 to 90 per cent of the possible range of splenic reduction was covered by frequencies up to 2 impulses per second — that is, rates well within the 'low physiological frequency region' of sympathetic nerve fibre discharge.

It might be questioned if the identification and isolation of sympathetic nerve fibres in the splenic pedicle were a complete one. The isolation of the greater and lesser splanchnic nerves to the left

adrenal is much safer in that respect. Further, if some splenic nerve fibres remained undetected and intact, these fibres, as well as those innervating the left adrenal gland, would be excited when the left splanchnic nerves were stimulated. This is quite possible as the praeganglionic nerve fibres to the spleen are to be found in the splanchnic nerves (see e. g. UTTERBACK 1944/45), and might cause a disturbing artifact reinforcing the effects of the medullary output. The general trends of these experimental difficulties are to decrease the relative importance of the constrictor nerves. This would imply that, in reality, the constrictor nerve control might be still more prominent as compared with that of the adrenal medullary hormones.

A regularly observed phenomenon, which demands some comment, was that the medullary hormones, whether released from the adrenals or infused intravenously, did not cause such marked *maximum* constrictions as did stimulation of the splenic nerves. A tentative explanation is the following. Stimulation of the splenic nerves will contract the smooth muscle cells of the splenic blood vessels, as well as those of the capsule and trabecular framework and thereby strongly decrease splenic blood flow. The release and action of the adrenergic transmitter, liberated locally at the splenic nerve endings, is probably not primarily affected by a reduction of blood flow. On the other hand, the admittance to the spleen of any *blood-borne* constrictor substances is to some degree 'self-limiting', as it is a function of the rate of blood flow. Consequently, the contractions of the spleen, caused by the blood-borne medullary hormones, will be limited by the concomitantly induced reduction of splenic blood flow.

Numerous investigators (see e. g. OLIVER and SCHÄFER 1895 and HOSKINS and GUNNING 1917) have pointed out the great sensitivity of the spleen to adrenaline. The demonstration of noradrenaline in the spleen and splenic nerves by EULER (1946, a, b and c) and the postulated identity between 'sympathin E' and noradrenaline, EULER (1948) raised curiosity as to the action of noradrenaline on the contractile elements of the splenic tissue. The constrictor action on spleen of adrenaline and noradrenaline was studied in the cat and the dog by HOLTZ, BACHMANN, ENGELHARDT and GREEFF (1952 b). They concluded that in both animals adrenaline was the stronger constrictor agent. Especially in the dog the predominance for adrenaline was pronounced, noradrenaline

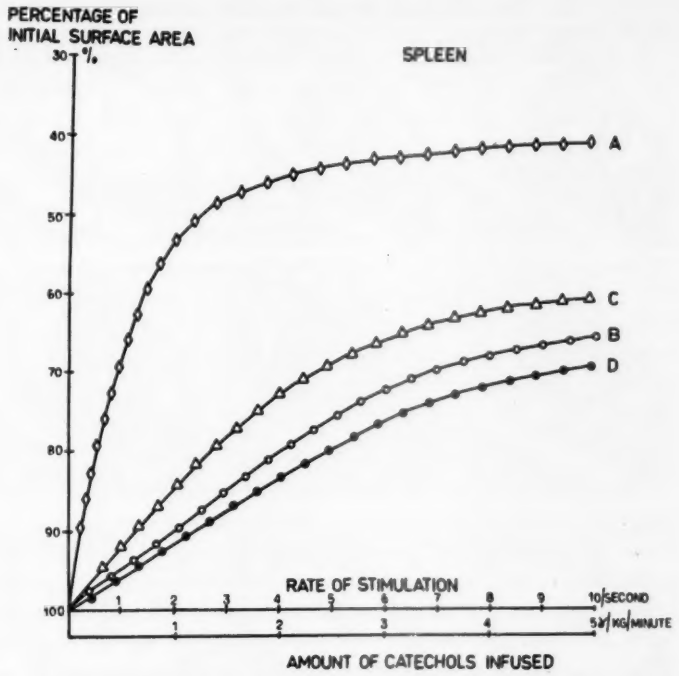

Fig. 20. Control of spleen size by sympathetic splenic nerve fibres (curve *A*) and *both* adrenal medullae (curve *B*) — upper abscissa. Curves *C* and *D* show the effects of l-adrenaline (curve *C*) and l-noradrenaline (curve *D*) — lower abscissa.

being four to five times less active. Their results are in agreement with those demonstrated on the cat in the present study.

The diagrams in fig. 20 summarize the contractions of the spleen obtained in this study in response to (*A*) stimulation of the splenic nerves, (*B*) the adrenal medullary discharge (this curve is calculated to present the responses to activation of *both* adrenal glands — see p. 47), (*C*) l-adrenaline and (*D*) l-noradrenaline. The predominance of the direct sympathetic innervation in control of splenic size is marked when the response, at a given frequency of splenic nerve stimulation, is compared with that in response to the adrenal medullae when activated at a similar rate.

E. The iris and the nictitating membrane

So far this study has been concerned with some smooth muscle effector groups of great importance in the control of peripheral circulation. Evidence has been presented to show, that, regarding the motor control of these effectors, this is quite dominated by the corresponding sympathetic innervation. It might be that this predominance of the direct sympathetic constrictor fibres, in comparison with the adrenal medullae, is valid only in the control of blood vessels. In order to see if the abovementioned relationship is a more widely applicable one to effectors subordinate to the sympathetic nervous system, the responses of the smooth muscle cells of the nictitating membrane and, the dilator muscle of the iris, were included in this study.

Method

The responses of the nictitating membrane were recorded in eleven and those of the iris in seven cats. For the recording of the contractions of the nictitating membrane see Chapter II (p. 21). The magnitude of the pupillary dilatations was measured from photographs. The head of the cat was fastened in a holder and the eyelids were tied aside. To prevent drying of the cornea the eye was irrigated with saline at short intervals. The photographs were taken at a close distance which was kept constant.

The corresponding cervical sympathetic trunk was carefully isolated proximal to the superior cervical ganglion and placed on an Ag-AgCl-electrode. Activation of the left adrenal gland was achieved as in the previous series on different vascular regions. Intravenous infusions of l-adrenaline and l-noradrenaline were performed through the left brachial vein. There was no attempt to keep the 'perfusing' blood pressure to the head constant in this series.

Results

The dilator muscle of the iris

The pupil was markedly expanded by low frequency stimulation of the corresponding sympathetic trunk. As a matter of fact in three out of seven experiments of this kind *each maximal single shock*

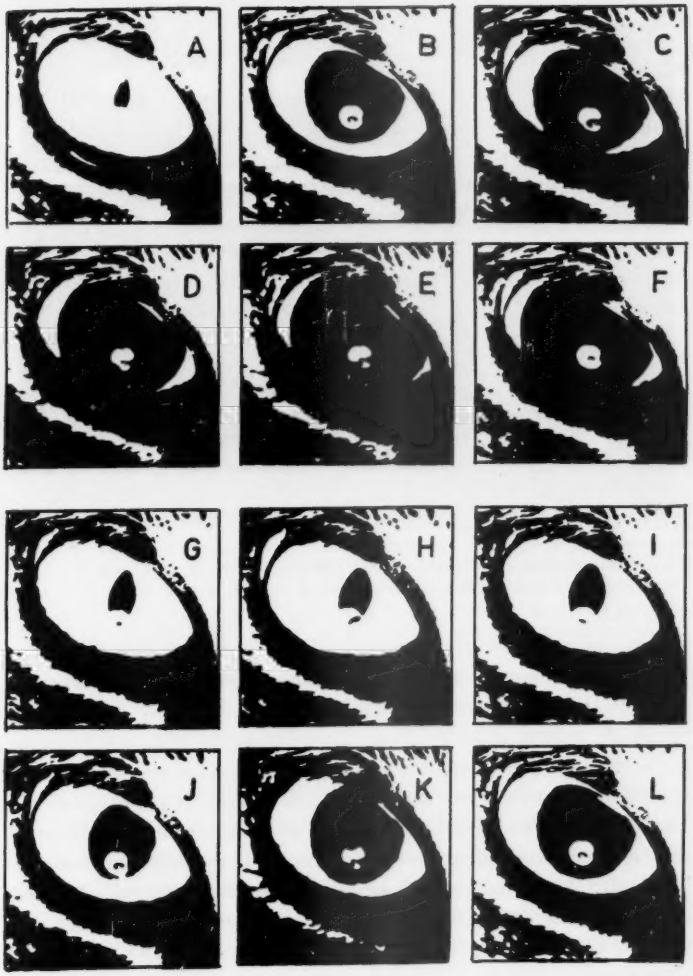

Fig. 21. Iris. Dilatations induced by the sympathetic innervation (*B*—*F*) and the adrenal medullary discharge (*H*—*L*). *A* — Control, *B* — Stim. cervical sympathetic trunk (30/min), C — Do. (1/sec), *D* — Do. (2/sec), *E* — Do. (4/sec), *F* — Do. (8/sec), *G* — Control, *H* — Stim. left splanchnic nerves (2/sec), *I* — Do. (4/sec), *J* — Do. (8/sec), *K* — Do. (16/sec), *L* — Do. (32/sec).

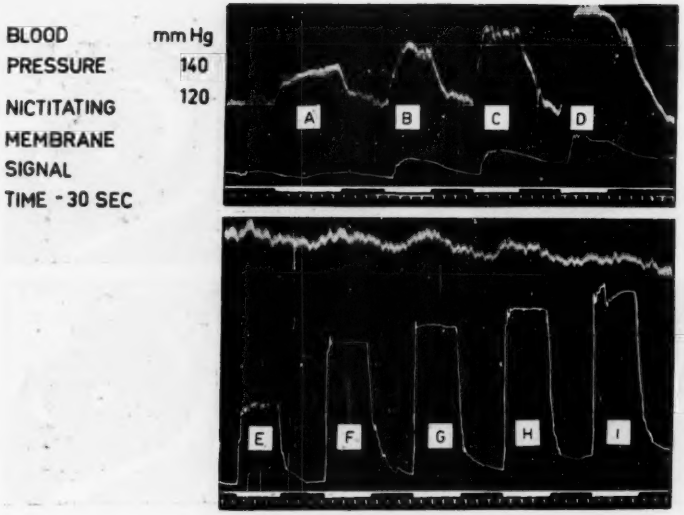

Fig. 22. Nictitating membrane. Effects of the adrenal medullary discharge ($A-D$) and stim. of the cervical sympathetic trunk. A — Stim. left splanchnic nerves (2/sec), B — Do. (4/sec), C — Do. (8/sec), D — Do. (16/sec), E — Stim. cervical sympathetic trunk (30/min), F — Do. (1/sec), G — Do. (2/sec), H — Do. (4/sec), I — Do. (8/sec).

stimulus produced an almost maximal dilator response. This dilatation of the pupil was not sustained until the rate of stimulation was too rapid as to allow a return to initial state in the intervals between the single volleys of stimulation. At a frequency of 2 to 4 impulses per second the pupil expanded almost to maximum and higher rates of stimulation added comparatively small changes of pupillary width. The contractions of the dilator muscle of iris in response to stimulation of the corresponding cervical sympathetic trunk is demonstrated in fig. 21. Again the adrenal medullary discharge proved much less effective in its action upon the iris and the stimulation of the left splanchnic nerves had to be performed at a rather high rate in order to produce a definite dilatation of the pupil (see fig. 21).

The pupillary dilatation accompanying intravenous infusions of l-adrenaline and l-noradrenaline was not very pronounced until the rate of infusion was a fairly high one. Of the two adrenal catechols l-noradrenaline proved distinctly less effective than l-adrenaline.

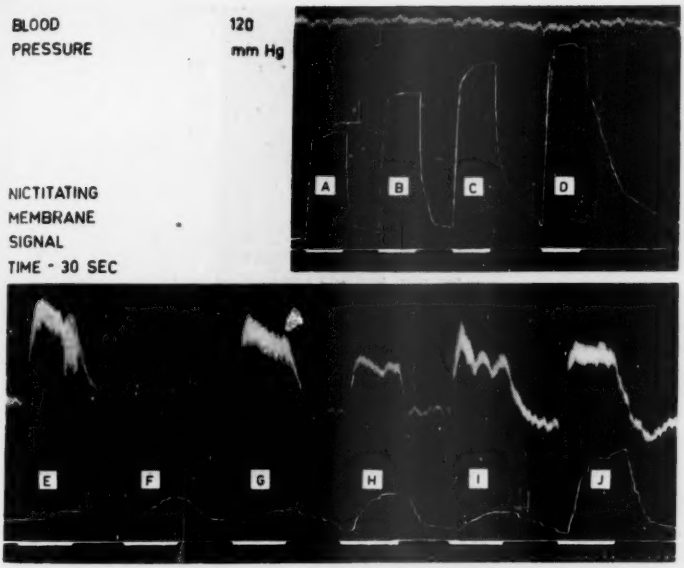

Fig. 23. Nictitating membrane. Effects of stimulation of the cervical sympathetic trunk (*A* — *D*) and infusion of l-adrenaline and l-noradrenaline (*E* — *J*). *A* — Stim. cervical sympathetic trunk (30/min), *B* — Do. (2/sec), *C* — Do. (8/sec), *D* — Do. (16/sec), *E* — I. v. infusion l-noradrenaline (1 μg/kg/min), *F* — Do. l-adrenaline (1 μg/kg/min), *G* — Do. l-noradrenaline (2 μg/kg/min), *H* — Do. l-adrenaline (2 μg/kg/min), *I* — Do. l-noradrenaline (3.6 μg/kg/min), *J* — Do. l-adrenaline (3.6 μg/kg/min).

The nictitating membrane.

The profound difference as to the relative effectiveness of the direct sympathetic innervation, on the one hand, and the adrenal medullary hormones on the other in the motor control of this effector is evident from the experiments referred to in figs. 22, 23, 24. As seen from these figures the contractions of the membrane were almost maximal when the corresponding sympathetic nerve fibres were excited at rates about 2 to 4 impulses per second, and higher rates added only small increases to these effects. When compared with these rather extensive contractions the responses to activation of the left adrenal (fig. 22) or to the infusion of catechols (fig. 23)

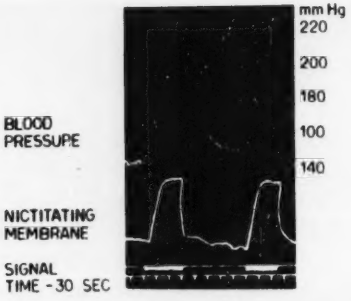

Fig. 24. Nictitating membrane. *A* — Stim. left splanchnic nerves (10/sec) and — *concomitantly* — stim. cervical sympathetic trunk (30/min), *B* — Stim. cervical sympathetic trunk only (30/min).

— even up to definitely 'nonphysiological' amounts — were of minor importance. In other experiments the membrane altogether failed to contract following stimulation of the left adrenal, even though low frequency stimulation of the cervical sympathetic trunk provoked extensive contractions. It might be that 'subthreshold' amounts of the medullary catechols, although ineffective by themselves, are capable of increasing the contraction of the membrane induced by stimulation of the cervical sympathetic. To test this possibility activation of the left adrenal and of the direct sympathetic innervation to the membrane was carried out simultaneously (see fig. 24). If the splanchnic stimulation was performed at 10 impulses per second and *at the same time* the cervical sympathetic was stimulated at 1 impulse every other second there was a blood pressure rise and in addition a moderate contraction of the membrane. However, a contraction of a similar magnitude was obtained in response to stimulation of the cervical sympathetic alone at a rate of 1 impulse every other second. The view that the adrenal medullae might reinforce the action of the direct innervation was not supported by these experiments on the nictitating membrane. As seen from fig. 23 (E, G, I) l-noradrenaline is rather ineffective in its action upon the nictitating membrane, and it was a regular finding in this short series that l-noradrenaline had to be administered in amounts some 4 to 8 times those of l-adrenaline in order to evoke a similar response.

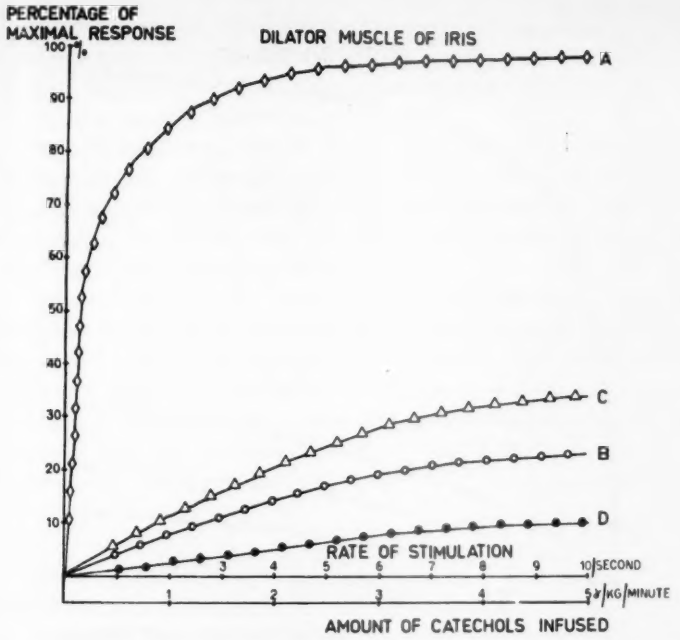

Fig. 25. Control of the dilator muscle of iris by the direct sympathetic innervation (curve *A*) and *both* adrenal medullae (curve *B*) — upper abscissa. Curves *C* and *D* show the effects of l-adrenaline (curve *C*) and l-noradrenaline (curve *D*) — lower abscissa.

Comments

The iris has a dual innervation receiving sympathetic fibres which contract the dilator muscle and parasympathetic fibres which contract the constrictor muscle. In the present study the effects upon stimulation of the sympathetic fibres were recorded whereas the parasympathetic fibres were left intact. The expansions of the pupil thus effected will induce a reflex increase of the tonic activity of parasympathetic nerve fibres acting upon the constrictor muscle, because more light is now falling on the retina. That means that the contractions of the dilator muscle following sympathetic stimulation is opposed by the constrictor muscle. If, however, atropine was given to block the peripheral actions of the cholinergic nerve

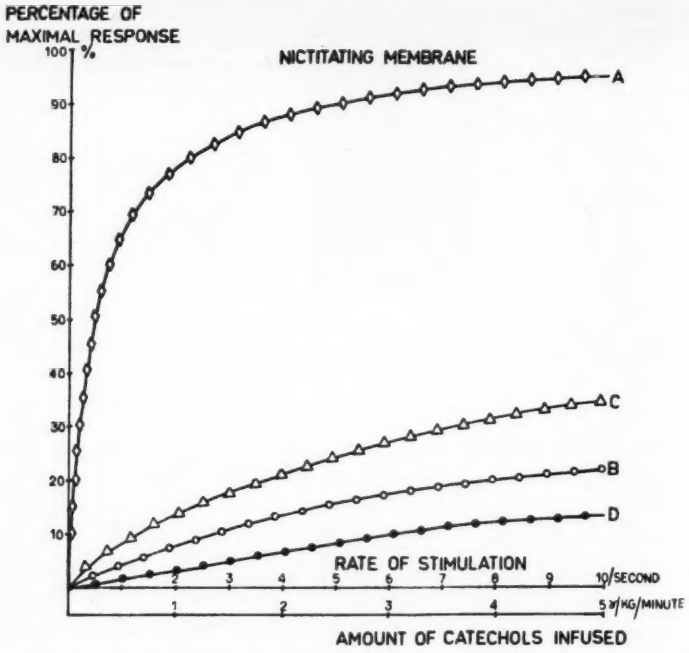

Fig. 26. Control of the nictitating membrane by the direct sympathetic innervation (curve *A*) and *both* adrenal medullae (curve *B*) — upper abscissa. Curves *C* and *D* show the effects of l-adrenaline (curve *C*) and l-noradrenaline (curve *D*) — lower abscissa.

endings in the constrictor muscle the iris dilated markedly even if the sympathetic innervation of the antagonist was centrally cut. It was decided not to use atropine because the dilated iris is not in a good position to reveal any dilatation induced by the sympathetic nerves or by the medullary hormones. Further, the limiting influences of the light reflex upon the dilator responses will be a fairly constant experimental error and not dependent on whether the dilatation is effected by the sympathetic innervation or by the adrenal medullary hormones. It will thus not impede a comparison of the relative effectiveness of the two components. However, the light reflex might be responsible for the rapid return of the iris to the initial state that was regularly observed when the rate of sym-

pathetic stimulation was below 1 impulse per second. But, in spite of the restricting action of the constrictor muscle low frequency stimulation of the cervical sympathetic proved quite effective in eliciting a maximal dilatation of the pupil.

The responses of the dilator muscle of the iris to (*A*) stimulation of the cervical sympathetic, (*B*) the adrenal medullary discharge (both adrenals), (*C*) l-adrenaline and (*D*) l-noradrenaline are summarized in fig. 25. Fig. 26 presents a similar summing up of the responses of the nictitating membrane.

It is obvious that these two effectors do not differ to any marked extent from the blood vessels as regards their sympathetic control. Stimulation at a low frequency well within the physiological discharge range of sympathetic nerve fibres will produce maximal responses. Moderate contractions were obtained in response to the adrenal medullary discharge but then only if the adrenals were activated at the top of their capacity.

Discussion

The aim of the experiments reported in this chapter was to present an approximate quantitative estimate regarding the possible range of control on various smooth muscle effector groups exerted by the two efferent links of what has been called the 'sympathico-adrenal system'.

Comparing the responses obtained by stimulation of the direct sympathetic innervation, on the one hand, and those brought about by way of the adrenal medulla on the other, all effectors studied appear to have some characteristics in common.

Regarding the maximum responses possible to provoke by separate stimulation of the two components at the top of the 'physiological' discharge range of sympathetic nerve fibres, it was found that those evoked by way of the direct sympathetic innervation were by far more intense than those evoked by way of the adrenal glands. That is to say that the full range of motor control of the direct innervation is considerably wider than that of the medullary hormones.

Secondly, if a comparison is made between the effector responses

following separate activation of the two components at one and the same low range frequency, the predominance of the direct innervation is still more prominent. At frequencies which can be considered as low or moderate for the sympathetic nervous system — e. g. up to 2 impulses per second — it is possible to bring about very pronounced responses of most effector systems by way of their sympathetic fibres. For example, note the constriction of skin vessels to *1 impulse every fourth second* (fig. 13), the marked splenic shrinkage (fig. 18) and the responses of the iris and nictitating membrane (figs. 21, 22, 23). In order to get responses of a similar magnitude by maximal stimulation of the two adrenal glands they had to be excited at rates some five to twenty times higher. This further stresses the predominance of the direct sympathetic innervation.

When the widely distributed sympathetic fibres to the different effector groups and the adrenal medullae are brought together under the common heading 'the sympathico-adrenal system' a marked co-operation between these two components, as to their effects on subordinate tissues, has been commonly understood, and much stress seems often to be laid upon the quantitative importance of the adrenal medulla. There is good evidence that sympathetic motor fibres as well as the secretory nerves to the adrenal medulla are controlled by central structures at both bulbar, hypothalamic and cortical levels. Changes of the internal or external environment of the individual will modify the activity of these central structures and thus the discharge of fibres in the thoraco-lumbar sympathetic outflow. Some of those changes will probably engage a certain group of the neuroeffector units separately, while others will mainly excite only the adrenal medulla. For instance, exposure to moderate cold will increase in the first place the activity of the sympathetic vasoconstrictor fibres to the skin vessels. A moderate fall in blood sugar level on the other hand might excite predominantly the cells of the adrenal medulla. Other changes, however, such as most types of general stress, work, emotional excitation etc., will cause a more or less generalized increase of sympathetic activities, involving both sympathetic motor nerve fibres and the adrenal medulla. For these diffuse activation patterns CANNON coined the phrase 'emergency reactions' and the adrenal medullary

secretion has been called the 'emergency hormone'. The morphological arrangement of the sympathetic nervous system with a marked divergence and convergence principle, both regarding the distribution of the prae- and postganglionic fibres, is in accordance with this type of general and diffuse activation.

However, a reinforcement of the motor control by the sympathetic postganglionic nerve fibres, effected by way of the adrenal medullary discharge, is ultimately a question of the relative effects which the two components are able to induce on one and the same effector. If this point of view is applied, it seems improbable that the role of the adrenal medulla in the homeostatic adjustments of the smooth muscle effector groups studied, is an important one. It should be kept in mind that in the present study, the initial position of the adrenal glands in this comparison was somewhat favoured. Firstly, the splanchnic stimulation will activate *all* cells whether they produce adrenaline or noradrenaline. There are, however, good reasons to accept a functional differentiation of the adrenal medulla and that adrenaline or noradrenaline often might be secreted separately by different cells. See especially the papers of HILLARP (1946), BEAUVALLET, BRETON and SALLE (1951), EULER and FOLKOW (1953) and HILLARP and HÖKFELT (1953). Thus the adrenal medullary discharge might consist of mainly adrenaline or mainly noradrenaline. An intense reflex activation of all cells probably occurs only in states of extreme 'emergency'. A medullary release up to 5 μg/kg/min, therefore, is probably not met with in the living body. This amount rather represents a general estimate of the theoretically maximal possible capacity regarding the secretion of catechols from the adrenals. Secondly, the constrictor action of these hormones constitute another 'brake-mechanism' because the admittance of these blood-borne substances to different tissues is a function of local blood flow. Further, the local blood flow will be frequently reduced by the action of sympathetic constrictor nerves. On the other hand the release of the adrenergic transmitter is a local one and not primarily dependent on blood flow.

Apart from the idea of a reinforcement of the direct sympathetic innervation in the control of various smooth muscle effector groups, generally ascribed to the adrenal medulla, the medullary hormones are known to have some 'metabolic effects'. The most prominent of these

include the mobilization of glucose from the hepatic glycogen and the break-down into lactic acid of glycogen stored in the skeletal muscles. The 'metabolic' actions occur in tissues most probably not under a direct control of sympathetic fibres. In case of the liver the sympathetic innervation of the blood vessels is well established. The role of hepatic nerves in the carbohydrate metabolism is a matter of dispute but the general evidence indicates that the functions of the liver in carbohydrate metabolism are not effected by nerve impulses on liver cells. ALEXANDER (1940) in studying this matter could trace no nerve fibres into the parenchyma of liver lobules. For further references see e. g. LONG (1940) and KUNTZ (1945). The hyperglycaemia observed at various states of increased sympathetic activity is more or less totally abolished by adrenalectomy. See e. g. OLMSTED (1925), MIKAMI (1926) and JAEGHER and VAN BOGAERT (1935).

Concerning the skeletal muscles there are conflicting opinions about the existence and functional importance of a sympathetic innervation to the skeletal muscle cells themselves. On the whole evidence seems to strongly contradict these possibilities. For a fuller discussion see KUNTZ (1945). In any case the break-down of glycogen in skeletal muscle cells seems to be poorly effected by sympathetic nerve stimulation to skeletal muscles. As it seems improbable that the muscle cells themselves receive sympathetic nerve fibres, any glycogenolytic action of sympathetic nerves must be attributed to the transmitter released at vasoconstrictor nerve endings. In comparison to the ease with which the break-down of glycogen is executed by the medullary output, the importance of the direct sympathetic innervation in this respect must be small.

As stated previously, the 'motor' functions of the medullary hormones are insignificant compared with the motor functions of the direct sympathetic innervation. As regards the 'metabolic' functions of the competitors the reverse seems to be true. These metabolic changes occur, as mentioned, in tissues most probably devoid of a sympathetic innervation and thus depend on a generalized distribution of the medullary hormones. Another point in favour of the view that the main importance of the adrenal medullary hormones is to be looked for primarily in the field of their metabolic actions rather than in the motor control of smooth muscle effector groups, is the fact that these metabolic effects are pronounced at a very

low concentration of adrenaline compared with those that are able to induce contractions of smooth muscles. Noradrenaline is much less potent than adrenaline as regards the 'metabolic' effects. See e. g. LUNDHOLM (1949 a), SCHÜMANN (1949) and BEARN et al. (1951).

The dilator action of adrenaline on muscular blood vessels at first glance does not seem to fit the general picture of the sympathetic control of the smooth muscle effector groups studied. First, the effect is here a relaxation of the vascular smooth muscles and further, this dilator response reaches a maximum at concentrations of adrenaline which are much lower than those exerting a definite constrictor action on e. g. the very sensitive skin vessels. Maximal dilatations could be obtained by amounts well within the capacity of the adrenal medullae. In addition the concentrations of adrenaline which induce dilatations seem to be of the same order of magnitude as those which cause glycogenolysis and lactic acid production. LUNDHOLM (1951) has suggested that this dilator action of adrenaline is an indirect one and caused by the released lactic acid. The results of the present study regarding threshold concentrations for on the one hand constrictor effects and for the dilator response of muscular blood vessels on the other seem to conform with this theory. It is of some interest to notice that this dilatation of muscular blood vessels brought about by adrenaline is probably not counteracted by the constrictor nerves. If adrenaline is secreted in amounts that dilate the blood vessels of skeletal muscles but, as a *net* effect produce an elevation of the blood pressure, this blood pressure rise will *via* the baroreceptor regions, effect an inhibition of the constrictor fibre discharge and thus further dilate blood vessels. The unique position of the blood vessels of the muscles as to their sympathetic control is also evident from the existence of a sympathetic cholinergic vasodilator innervation of these vessels. By co-operation these dilator fibres, adrenaline and the metabolites produced by muscular activity all may oppose the restricting influences of the vasoconstrictor nerves. In states of 'emergency' they may form the background of a 'borrowing and lending mechanism' by which an adequate blood supply to the skeletal muscles is secured.

Thus, the dilator action of adrenaline fits the scheme of the 'emergency hormone' as elaborated by CANNON. There are, as mentioned, reasons to belive that the dilatation of muscular blood vessels

is ultimately a consequence of the glycogenolytic action of l-adrenaline. In that case the break down of glycogen into lactic acid by l-adrenaline would furnish energy for muscular work and at the same time increase blood flow through skeletal muscles.

In the present study a comparison of the motor responses to l-adrenaline and l-noradrenaline was included. The general rule was that the constrictor action of l-adrenaline on these smooth muscle effector groups was more pronounced than that of l-noradrenaline. The ratio of 'equiconstrictor' doses of l-noradrenaline and l-adrenaline (N/A) varied on different substrates. It was in some cases of the magnitude of 2 to 8/1, e. g. skin vessels and nictitating membrane. That is, l-noradrenaline had to be administered in amounts 2 to 8 times that of l-adrenaline in order to obtain the same response.

In this connection some comments might be given on certain aspects of the effects of the catechols on the circulatory system as a whole. As seen from the experiments reported in this study l-noradrenaline — when administered at low concentrations — effected only minor changes in peripheral resistance of the decentralized vascular regions studied (see e. g. the diagrams in figs. 9, 14, 17 and 20). In contrast to this it was found in a small complementary study (comprising nine animals made spinal) that intravenous infusions of l-noradrenaline at a low dosage had a rather marked capacity to raise arterial blood pressure. So for instance 0.4 μg/kg/min quite regularly brought about a sustained elevation of the spinal blood pressure to about double the initial level. As seen from figs. 9, 14, 17 and 20, on the other hand, the increases of peripheral resistance of the decentralized vascular regions at a similar dosage of l-noradrenaline were rather insignificant. A possible mechanism, by which this apparent paradox might be explained, is that the rise of the general blood pressure in the spinal animal is only partly due to an increase of the peripheral resistance. According to the Poiseuille formula (Pressure = C · Resistance · Flow) changes of the arterial blood pressure might also be attributed to variations in the cardiac output. It should be kept in mind that the administration of catechols will affect the heart muscle itself and also, most probably, the smooth muscle cells of the venous vessels. Thus, the direct excitatory influence of l-noradrenaline on the ventricles might effect a more

complete emptying of the ventricular residual blood and thereby increase cardiac output. Especially as in the spinal cat all nervous regulatory mechanisms are ruled out. Further, an extremely slight constrictor action on the venous tree will mobilize a rather significant extra venous return to the heart. Some quantitative considerations might be given as an illustration without putting stress on the actual figures given. If one considers that each heart beat puts out 2 ml. of blood in a cat of 3 kg of body weight and that 60 per cent of the total blood volume is contained on the venous side, that is roughly 150 ml., a doubling of the venous return is obtained if 2 ml. of the blood in the veins is pushed towards the heart. This is obtained by only a 1 per cent shortening of the smooth muscles in the venous walls, while a similar shortening of the smooth muscles of the small vessels responsible for the peripheral resistance will increase the resistance only about 4 per cent. Thus, small amounts of catechols, which only slightly affect the vascular smooth muscles and the peripheral resistance, might considerably increase the cardiac output. This example is another illustration that the blood-pressure record alone is a quite unreliable indicator on changes of the *peripheral resistance*, and no quantitative conclusions concerning the actual haemodynamic adjustments behind the blood-pressure rise should be drawn from such experiments.

The possibilities here considered might have a bearing upon the effects of vasoactive therapeutics, the action of which, in many respects, are rather poorly understood.

CHAPTER IV

Some observations on the occurrence and effects of circulating 'sympathin'

In the following account the term 'sympathin' is used to imply the substance or substances that are released at the adrenergic nerve endings and which might diffuse into the blood stream when the fibres are activated (see CANNON and BACQ 1931).

In a review of the literature concerning reflex liberation of 'sympathin' ROSENBLUETH (1950) concluded: '*The importance of sympathin as a hormone with generalized action, which co-operates with adrenaline is thus not negligible for ensuring diffuse sympathetic action*' (the italics are the present author's).

The present study is an attempt to answer the following questions:

(i) Which are the requisites that allow the demonstration of 'sympathin' in the circulating blood and how big can its concentration be?

(ii) Are these concentrations high enough to elicit any quantitatively significant effects as compared with the effects of the direct innervation and the adrenal medullary discharge?

Method

In order to study the possible existence and approximate concentrations of circulating 'sympathin' experiments were performed on eighteen cats anaesthetized as previously described. Additional observations were made on the animals used for the determinations of the magnitude of the adrenal medullary secretion referred to in Chapter II.

The release of 'sympathin' was effected by maximal stimulation of the peripheral ends of the two abdominal sympathetic chains (supplying a tissue amount of some 25 to 30 per cent of body weight),

one of the cervical sympathetic trunks, the splenic nerves and the renal nerves. As an indicator for circulating 'sympathin' the nictitating membrane of the same animal was used. The membrane was made supersensitive to 'sympathin' by extirpation of the corresponding superior cervical sympathetic ganglion about two weeks prior to the experiment.

The intestines and the great omentum were extirpated. Reflex activation of the adrenal medullae was eliminated as previously. Most of the animals in this series were made spinal by transection of the medulla at the height of the sixth cervical segment. Artificial respiration was applied throughout the experiments. The spinal transection will release the thoraco-lumbar sympathetic outflow from the tonic control and reflex influences induced by way of centres at higher levels, and thus prevent a 'basal' release of 'sympathin'. The experiments were not executed until the blood-pressure rise that accompanied the transection of the spinal medulla had subsided and the blood pressure had stabilized at the 'spinal level' of about 60 to 70 mm Hg. The blood pressure was recorded from one of the brachial arteries. The lower abdominal sympathetic chains, the right cervical sympathetic trunk, the splenic and the renal nerves were isolated and prepared for stimulation as previously described (see Chapter III). The preparation of the denervated nictitating membrane is described on p. 21. l-Adrenaline and l-noradrenaline were injected or infused at a constant rate either intravenously through one of the brachial veins or into the abdominal aorta through the inferior mesenteric artery. In some animals 10 mg cocaine per kg of body weight was given by slow intravenous infusion. The possible influences of circulating 'sympathin' on normal effectors — without the aid of any sensitizing procedure — were investigated in an additional series of eighteen cats. The sources of 'sympathin' were the same as when studying the remote effects on the denervated nictitating membrane. The responses of muscular blood vessels, skin vessels, renal vessels, spleen and the nictitating membrane were recorded as previously. These effectors were deprived of their sympathetic nerves and reflex release from the adrenal medullae was prevented as previously described. To eliminate the tonic and reflex influences of the sympathetic with a possible release of sympathin as a conse-

quence some of the animals were made spinal. Thus, if there were any response of the effector studied this could be attributed to the release of 'sympathin' accompanying the sympathetic stimulation of other effectors.

Results

In most animals l-adrenaline or l-noradrenaline given intravenously at a dosage of 0.1 to 0.2 μg produced a small but definite contraction of the denervated nictitating membrane, demonstrating an acceptable sensitivity to these catechols. It was easy to check whether the electrical stimulation of the various sympathetic structures mentioned above was effective by observing the marked local responses of the effectors innervated. For instance, stimulation of the lower abdominal sympathetic chains was obviously effective when the fur of the tail and lower part of the back was erected by the activation of the piloerector muscles and the blood pressure rose some 40 to 60 mm Hg because of the constriction of blood vessels in the hind limbs. However, in spite of the fact that the nictitating membrane contracted when minute doses of the catechols were injected intravenously and, in spite of the fact that the stimulation of the sympathetic fibres to various effectors proved highly effective to judge from their local responses, in many of the experiments there occurred no definite contraction of the denervated nictitating membrane at all. In no experiment did the denervated membrane contract in response to stimulation of the contralateral cervical sympathetic trunk or to stimulation of the sympathetic renal nerves. In a few animals a minimal response occurred upon stimulation at high rates (20 to 40 impulses per second) of the splenic nerve fibres, but then only if cocaine had been given previously. In some animals stimulation of the lower abdominal sympathetic chains was followed by a small contraction of the denervated nictitating membrane. In order to achieve such a response the rates of stimulation of the sympathetic chains had to be fairly high and, in addition the indicator had to be utterly sensitive to l-adrenaline and l-noradrenaline. A typical illustration of these observations is given in fig. 27. It should be noticed that in this animal the intravenous injection of 0.05 μg of l-adrenaline produced a definite contraction of the denervated nictitating membrane indicating a pronounced respons-

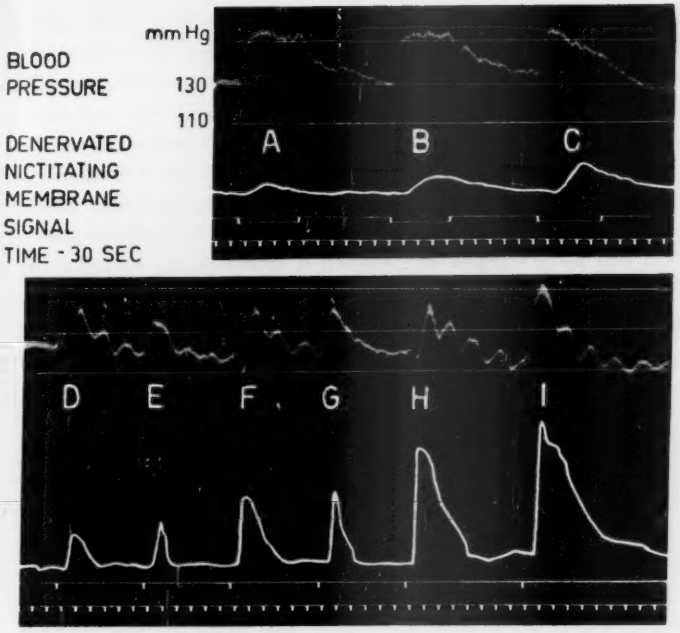

Fig. 27. Effects of 'sympathin' on the denervated nictitating membrane. *A* — Stim. lower abdominal sympathetic chains (5/sec), *B* — Do. (10/sec), *C* — Do. (20/sec), *D* — I. v. injection l-adrenaline (0.05 μg), *E* — Do. l-noradrenaline (0.05 μg), *F* — Do. l-adrenaline (0.1 μg), *G* — Do. l-noradrenaline (0.1 μg), *H* — Do. l-adrenaline (0.5 μg), *I* — Do. l-noradrenaline (0.5 μg).

iveness of the indicator. If now the sympathetic chains were stimulated at a low frequency there was no response whatsoever of the nictitating membrane. At a rate of 5 impulses per second continued for two minutes there was a small 'remote effect' on the membrane which could be matched by minute amounts of catechols and, which, most probably, could be attributed to 'sympathin' entering the blood stream. This 'remote effect' was more prominent and, further, somewhat prolonged, if the rate of stimulation was increased up to 10 and 20 impulses per second.

In some animals cocaine was administered intravenously at a dosage of 10 mg per kg of body weight. Cocaine in this amount is known to facilitate the release of 'sympathin' into the blood stream

and to induce a supersensitivity of various effectors to the action of the adrenal catechols and also to the action of 'sympathin'. However, in these experiments the nictitating membrane was already made supersensitive by previous denervation and this supersensitivity, as tested by catechols, was not much altered by the action of cocaine. On the other hand, the peripheral effects of sympathetic stimulation seemed to be strengthened. For instance, the rise of blood pressure, accompanying stimulation of the sympathetic chains, often was more marked and showed a slower return to the initial level after the injection of cocaine. In a few experiments the remote effects on the denervated nictitating membrane were increased but most trials to produce clear cut 'remote effects' still failed.

Not in a single case out of numerous trials in eighteen experiments was it possible to show even the slightest 'remote' effect on any type of acutely decentralized effector groups which could be attributed to the existence of circulating 'sympathin'. This was the regular finding in spite of the fact that the stimulation of various sources of 'sympathin' proved effective as regards the local responses and, in spite of the fact that the effector groups studied showed the normal range of responses to stimulation of their corresponding sympathetic innervation and to the adrenal medullary catechols.

These results appeared to indicate that even at an intense activation of the peripheral sympathetic nerve fibres only exceedingly small amounts of the transmitter substances will reach the general blood stream. Probably, the transmitter is locally inactivated at the site of the release. This hypothesis has often been claimed and was supported by the following observations. If l-adrenaline or l-noradrenaline was injected into the lower abdominal aorta by way of the cannulated inferior mesenteric artery fairly large amounts of these compounds had to be given in order to effect the denervated nictitating membrane. In some experiments the catechols were infused into the abdominal aorta at a rather high dosage, and the infusion was maintained until the nictitating membrane showed a moderate and sustained contraction. If there was no inactivation of the catechols by the tissues supplied by branches from the lower part of the abdominal aorta, the amounts of catechols in the venous outflow from these tissues must equal the amounts infused in the

steady state. Consequently, it would not make any difference at this moment if the infusion was shifted to an intravenous route. But, as a matter of fact, there was a pronounced increase in the response of the denervated nictitating membrane when this shift was performed. These phenomenons will be further investigated and discussed in a future study.

Comments

In the present study attempts to elicite definite 'remote effects' on the sensitized nictitating membrane due to circulating 'sympathin' released upon electrical stimulation of the sympathetic nerve fibres to various smooth muscle effectors generally failed or the effects were very slight.

It might be said, that perhaps this common failure was due to the fact that the indicator used was not sensitive enough to detect small amounts of 'sympathin'. Evidently any test has a threshold but in the case of the denervated nictitating membrane the responsiveness must be considered quite acceptable because in most animals the membrane contracted to 0.1 to 0.2 μg of l-adrenaline or l-noradrenaline when injected intravenously, and in some experiments the sensitivity of the membrane was still more pronounced (see e. g. fig. 27).

It might further be argued that the sympathetic stimulation of the effectors might not have been effective. This was, however, definitely not the case which could easily be checked by noticing the intense local responses of the effectors stimulated. There was quite regularly an erection of the hairs, a blood pressure rise, a paling of the renal surface, a shrinkage of the spleen and a pupillary dilatation depending on which region that was activated. Further, the sympathetic stimulation was carried out in exactly the same manner as described in Chapter III where the local responses of the effectors at different rates of stimulation were quantitatively recorded and found extensive.

In those experiments where a definite contraction of the denervated nictitating membrane occurred it was still very small and the stimulation of the lower abdominal sympathetic chains were executed at a rather high rate (5 to 40 impulses per second). These

rates are close to or beyond the upper limit of the physiological discharge range of sympathetic nerve fibres. In addition, the responsiveness of the indicator was marked. These contractions could be matched by minute doses of l-adrenaline or l-noradrenaline.

The common failure to induce any quantitatively significant 'remote effects', in spite of a rather sensitive indicator and, in spite of the fact that the sympathetic stimulation proved effective as regards the local response, appears to indicate that the release of 'sympathin' to the blood stream did not occur on a large scale. Further, even under circumstances where the stimulation was performed at supraphysiological rates, the amounts of circulating 'sympathin' were negligible when compared with the catechol discharge from the adrenals. This is true even if it is considered that the total amounts of 'sympathin' which might enter the blood stream from *all* tissues subordinate to the sympathetic nervous system should be some 3 to 5 times bigger. In some of the experiments approximate figures could be deduced which seemed to indicate that the 'overflow' of the transmitter is only around one twentieth of the secretion of the adrenal medullae when the stimulation rates are the same. Considering then that the adrenergic transmitter in the blood vessels is predominantly noradrenaline (SCHMITERLÖW 1948), and that the metabolic actions of this substance are not very prominent as compared with adrenaline, the amounts of circulating sympathin appear to be too small to elicit either 'motor' or 'metabolic' effects in the intact organism. Other authors claim that quite impressive amounts of 'sympathin' are liberated into the blood stream upon stimulation. See e. g. the papers of PEART (1949) and WEST (1950). According to PEART noradrenaline appeared in the venous outflow from the spleen following stimulation of the splenic nerves in amounts ranging from 0.05 to 0.5 μg per ml. plasma. Similar surprisingly high figures were reported by WEST. However, in their series cocaine was given and the rates of sympathetic stimulation were quite outside the physiological discharge rates of sympathetic nerve fibres. Further the determinations were made on blood samples withdrawn from the splenic vein and injected 'close-arterially' to the nictitating membrane or tested on isolated preparations. In the intact animal, on the other hand, the spleen

drains into the portal circulation. An enzymatic inactivation of the adrenergic transmitters by amine oxidase, present in the liver, has often been claimed (for references see e. g. BURN 1952 and BLASCHKO 1953) and might in part account for the general absence in the present series of 'remote effects' on the denervated nictitating membrane due to splenic 'sympathin'.

A more widespread reflex discharge of most components of the sympathetic outflow was induced in response to pain in adrenalectomized cats by LIU and ROSENBLUETH (1935) by stimulation of an afferent nerve. They concluded that this procedure *may* lead to a delayed contraction of the denervated nictitating membrane, but also made the following remark: '- - to demonstrate circulating sympathin the indicators have been rendered abnormally sensitive to the hormone by previous denervation or by cocaine. If these sensitizing procedures are not employed the effects of sympathin are small or absent'. Similar conclusions were put forward by MAGOUN, RANSON and HETHERINGTON (1937) who demonstrated the release of sympathin in response to hypothalamic stimulation using the denervated nictitating membrane as an indicator.

The claim by LIU and ROSENBLUETH, that the *acutely* denervated nictitating membrane, without the aid of cocaine, should be capable of a 'small' response to circulating 'sympathin' released upon stimulation of an afferent nerve, is somewhat surprising in view of the fact that its responsiveness to l-adrenaline is only one tenth to one twentieth of that of the *chronically* denervated membrane. The irresponsiveness towards l-noradrenaline is still more marked. (See p. 80).

In the present study clear cut contractions of the sensitized nictitating membrane occurred only in response to stimulation of the lower abdominal sympathetic chains. In these experiments, low frequency stimulation generally proved ineffective, whereas at higher (probably supraphysiological) frequencies the magnitude of the response was found to be roughly a function of the rate of stimulation applied to the sympathetic chains. The reason for this fact might well be that first when the amount of the transmitter in the blood stream has reached a certain 'threshold' level the indicator is able to respond. Another possibility, that has been claimed by FOLKOW (1952) is that at higher frequencies, above the physiological

discharge rates, the local elimination of the transmitter is no longer able to cope with the rate of release and that the 'overflow' is a consequence of this local accumulation. As a matter of fact, he could demonstrate that at frequencies below 6 to 8 impulses per second the time required for the relaxation of vascular smooth muscle cells was fairly constant. On the other hand, at higher frequencies (which exceed the physiological discharge range of sympathetic nerve fibres) the time required for relaxation rapidly increased indicating an accumulation of the transmitter. This phenomenon might correspond to the present observations concerning the appearance of 'remote effects'. The existance of a 'critical threshold frequency' has also been suggested by ROSENBLUETH and MORISON (1934).

As pointed out above, cocaine has frequently been employed by previous investigators to enhance the action of various sympathomimetic substances as well as 'sympathin' (see e. g. FRÖHLICH and LOEWI 1910, ROSENBLUETH and SCHLOSSBERG 1931, ROSENBLUETH and MORISON 1934 and BACQ 1937). The mechanism behind this action of cocaine is not settled.

In the present study cocaine was found to add little, if anything, to the sensitivity of an indicator already made supersensitive by previous denervation (see also e. g. ANITSCHKOW and SARUBIN 1928 and BURN and ROBINSON 1952).

The presence of the enzyme mono-amine oxidase in blood vessels has been demonstrated (see THOMPSON and TICKNER 1951 and COTZIAS, BALOURDAS and DOLE 1952). This enzyme is capable of inactivating adrenaline *in vitro*.

TORDA (1943) emphasised that the inactivation of adrenaline and 'sympathin' in the tissues was hampered if cocaine was added to the perfusing fluid. There is some evidence indicating that the concentration of this enzyme in different tissues is dependent, to some extent at least, on the presence of an intact sympathetic innervation (see e. g. BURN and ROBINSON 1952), and that the supersensitivity of a tissue towards sympathomimetic compounds accompanying degeneration of its sympathetic nerve fibres may be related to the extent of the fall in amine oxidase (see BURN and ROBINSON 1953). Cocaine has been shown to inhibit the inactivation of adrenaline by amine oxidase *in vitro* (see e. g. PHILPOT 1940). See

also the papers by GRUBER (1936), BACQ (1949) and the monograph by CANNON and ROSENBLUETH (1949), which give most of the literature on this topic.

The importance of these points are that they make up a plausible explanation why the failure to induce a definite 'overflow' of 'sympathin', as identified by a supersensitive indicator, often is turned into success after the administration of cocaine.

Another observation reported by THOMPSON (see p. 106 in *Visceral Circulation* 1952) which probably is of some importance in this connection indicates that the activity of amine oxidase is dependent on the prevailing oxygen tension. Even quite moderate reductions of the oxygen tension were claimed to markedly inhibit the enzyme activity. Now, if the sympathetic nerve fibres running to different effectors are maximally stimulated at very high rates the inevitable effect will be an intense vasoconstriction. The concomitant reduction of blood flow will lower the oxygen tension and might thus interfere with the enzymatic destruction of the transmitters. At the same time the transmitter substances might accumulate in abnormal concentrations due to the supraphysiological rate of stimulation. An 'overflow' of the adrenergic transmitter might then occur. The importance of adjusting the rate of electrical stimulation according to what could be expected to be met with during physiological conditions is a point commonly overlooked. With few exceptions previous trials to provoke an 'overflow' of the adrenergic transmitter have been carried out by sympathetic stimulation at supraphysiological rates.

Another observation in favour of the claim that the elimination of the adrenergic transmitter, at least partly, is effected by an enzymatic process at the site of release is reported in this study. If l-adrenaline or l-noradrenaline were infused into the abdominal aorta only a fraction of these catechols seemed to reappear in the venous blood, thus indicating quite heavy losses of the catechols during the passage through the tissues.

To summarize — the observation that only minute 'remote' effects were obtained on the nictitating membrane, although it was rendered abnormally sensitive, and then first when supraphysiological stimulation rates were applied and, further, the total irresponsiveness of acutely decentralized effector groups are facts which

strongly contradict the claim that circulating 'sympathin' should be capable of inducing any significant motor adjustments of effector groups subordinate to the sympathetic nervous system. Considering these quantitative aspects there seem to be poor reasons to characterize 'sympathin' as a hormone with generalized actions but it is probably more to be looked upon as an experimental curiosity.

CHAPTER V

The sympathetic response to asphyxia

The conclusions so far expressed in this study were based on a quantitative comparison of the effects obtained on different smooth muscle effector groups by electrical stimulation of the corresponding sympathetic innervation, on the one hand, and of the secretory nerves to the adrenals on the other. The stimulations were performed at different rates within the physiological discharge range of sympathetic nerve fibres. It would be of some interest, however, to compare the relative roles of these two components in a true homeostatic adjustment of the type that occurs in various states of 'emergency' or stress. In the experiments described in this chapter such a purely reflex activation of the sympathetic nervous system was brought about. The sympathetic influences on various smooth muscle effectors could be quantitatively recorded and attempts were made to evaluate the relative importance of the two efferent links of the so called sympathico-adrenal system in these adjustments.

Of the various stressful conditions, to which animals have been commonly exposed in order to provoke a widespread reflex excitation of the sympathetic nervous system, mainly three are applicable to the anaesthetized animal. Two of these, namely exposure to cold and the hypoglycaemia provoked by insulin, are relatively slow in their onset and protracted in their actions. They do not, therefore, suit the methods applied in this study. The third type in choice, which is asphyxia, differs in these respects. It is easy to provoke, it is fast in its onset and further, it can be repeatedly performed in one and the same animal. With some justification asphyxia might further be claimed to represent a quantitatively exaggerated combination of normal stimuli to the central structures governing the sympathetic nervous system.

It is of special importance that the stimulant should prove effective in eliciting a really intense activation of the adrenal medulla so that the medullary hormones will be released in amounts large enough to put the adrenals in a fair competitive position when a comparison with the direct sympathetic innervation is carried out. Extreme asphyxia appears to be safe in this very respect and, as a matter of fact, there is probably no other 'physiological' stressing which is more effective. See e. g. the papers by HARTMAN, MCCORDOCK and LODER (1923), SATO, INABA and TAKAHASHI (1932), RAPELA and HOUSSAY (1952), EULER and FOLKOW (1953) and REDGATE and GELLHORN (1953). At the same time, asphyxia is known to bring about important neurogenic cardiovascular effects, but, as far as I know, there is no safe knowledge about the relative roles of these two components in the asphyxial defensive reaction.

In the present study the following questions were raised:

(i) What is the magnitude of the total catechol output from the adrenals during asphyxia?

(ii) Which are the effects that this secretion will provoke on different smooth muscle effectors as compared with those effected by the corresponding direct sympathetic innervation?

The magnitude of the adrenal medullary discharge during asphyxia

Method

Experiments were performed on twelve cats anaesthetized as previously described (p. 20).

The indicator for the adrenal medullary hormones was the denervated nictitating membrane, made supersensitive by removal of the corresponding superior cervical sympathetic ganglion. This operation was performed under aseptic conditions about two weeks prior to the experiment. The arrangements for the recording of the contractions of the membrane in response to the medullary catechols are described on p. 21.

There are, as mentioned, principally two approaches for a quantitative determination of the adrenal medullary secretion. The one

involves a withdrawal of the adrenal venous outflow which is subsequently tested on its catechol content on various test preparations. Its main advantage is that it allows a quantitative parallel assay of adrenaline and noradrenaline. See especially EULER (1949), GADDUM (1950) and EULER and FOLKOW (1953). Another possibility, which was used in the present series, is to choose a test preparation within the experimental animal itself. Then there is no interference whatsoever with the circulation of the adrenals, and the response of the indicator will properly show the time sequence and the extent of the medullary excitation. If there are no attempts at differentiating adrenaline and noradrenaline, it follows that the indicator used has to be about equally sensitive to both these compounds. The denervated nictitating membrane appears to be one of the very few substrates to fulfil this requisite (see p. 24).

Arterial blood pressure was recorded from a femoral artery. l-Adrenaline or l-noradrenaline, or mixtures of both, were injected or infused into one of the brachial veins.

Asphyxia was induced by clamping off the tracheal cannula. It was maintained for different lengths of time, depending on the general condition and tolerance of the animal, generally 2 to 4 minutes. The asphyxia was discontinued when the blood pressure declined below 60 to 70 mm Hg and it was immediately followed by artificial respiration.

After two or three periods of asphyxia both adrenals were tied off. Thereafter the asphyxial stressing was repeated and the effects of the now eliminated adrenal medullary secretion on the denervated nictitating membrane were reproduced by an intravenous administration of catechols. Most probably the medullary discharge induced by the asphyxia is smooth in its onset, gradually reaches a peak and then slowly declines when artificial respiration is applied. Therefore, the substituting injection of catechols has to be given in a similar manner. EULER and FOLKOW (1953) determined the relative proportions of adrenaline and noradrenaline in the adrenal venous plasma of the cat during asphyxia and found that, as an average, about 75 per cent was noradrenaline. In the present series, therefore, the eliminated medullary secretion during asphyxia was substituted by injections of l-adrenaline and l-noradrenaline mixed in the proportions mentioned. Thus, it was possible, in most animals,

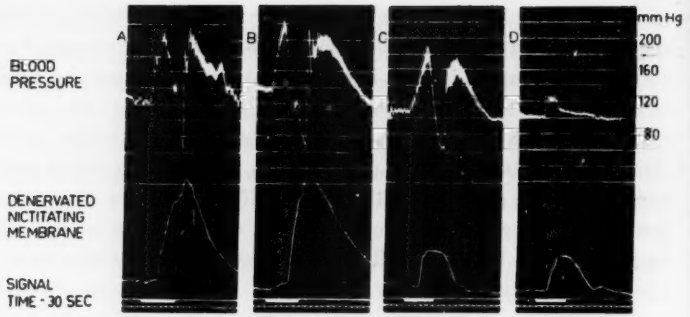

Fig. 28. Determination of the rate of catechol secretion during asphyxia. Body weight 3.5 kg. *A* — Asphyxia (3 min) — adrenals intact, *B* — Asphyxia (3 min) — adrenals extirpated but a substituting injection of catechols (in total 11.5 μg) gives the same response, *C* — Asphyxia (2 min, 30 sec), *D* — Injection of catechols (in total 0.6 μg). See text for further information.

to match fairly accurately the effects of the asphyxial medullary secretion on the denervated nictitating membrane, regarding time of onset, maximal height and duration of the response.

Results

As seen from fig. 28 asphyxia is accompanied by prominent circulatory adjustments. The large rhythmical variations that characterize the ascending limb of the blood-pressure curve coincided with increased respiratory efforts against closed airways. When they ceased to appear there was also a respiratory standstill which probably implied that the respiratory centre was too anoxic to function properly in spite of the combined excitatory influences of the accumulating carbon dioxide in the blood acting upon the centre and the reflex drive from the chemoreceptor regions. At this point the arterial blood pressure usually began to decline steadily, and the asphyxia would soon have been fatal unless artificial respiration was started. Evidence will be presented in the second part of this chapter to show that this decline of blood pressure is of cardiac origin and not due to a fall of the peripheral resistance. As soon as the asphyxia was interrupted there was a rapid rise in arterial blood pressure up to very high levels. The mechanism

behind this 'rebound phenomenon' is probably that the admittance of oxygen to the asphyxiated myocardium rapidly restores the cardiac output while at the same time the peripheral vasoconstriction is maintained.

It should be noticed that, only a few seconds after the period of asphyxia was started, there was a progressive rise of arterial blood pressure indicating an early excitation of the vasomotor centre. To judge from the responses of the sensitized nictitating membrane the adrenal medullae, on the other hand, appeared to be rather inactive at the beginning of asphyxia. Not until one and a half up to two minutes had passed a discharge of medullary catechols was observed as indicated by a powerful contraction of the denervated nictitating membrane. See *A* in fig. 28. *B* in the same fig. shows that after adrenalectomy approximately the same contraction of the membrane occurred if a substituting injection of a mixture of l-adrenaline (25 per cent) and l-noradrenaline (75 per cent) was given. The injection was not started until one minute of asphyxia had passed and was then performed at an increasing rate for two minutes and again slowed down during the third and last minute of injection. In total 11.5 μg was given during these three minutes with a maximum rate of about 2 μg/kg/min. This means that in this animal the rate of catechol secretion from the adrenals was as an *average* 1 μg/kg/min. The release of catechols during this period was most probably not uniform but moderate at the beginning and more intense at the height of asphyxia. As the contractions of the denervated nictitating membrane in the two instances were fairly equal (see *A* and *B* in fig. 28) the maximum rate of catechol release in this experiment was probably around 2 μg/kg/min. It should be remembered that the excitation must have been very strong as the asphyxia was extreme and most probably would have been fatal unless artificial respiration had been applied.

It is of interest that asphyxia in the adrenalectomized animal commonly evoked a small contraction of the sensitized nictitating membrane. This minute response was, at least partly, probably related to a release of 'sympathin' into the blood stream following the intense activation of the sympathetic nervous system. It could be matched by small amounts of catechols injected intravenously (see *C* and *D* fig. 28). However, if the spinal medulla was transected

in the lower cervical region, which was done in three animals, a slight contraction, though reduced, was still obtained in response to asphyxia in spite of the fact, that there was no blood-pressure rise indicating any major excitation of the now decentralized sympathetic nervous system.

The results described are representative for this series of experiments. It is natural that variations occurred both in a given experiment and when different animals were compared. In some animals the tolerance towards asphyxia was less than in the experiment referred to and the asphyxial medullary secretion was also less marked. In seven experiments, where the stressing of asphyxia could be maintained for 3 minutes or more, the rate of catechol secretion during this period averaged some 1.3 μg/kg/min with a maximal rate of secretion of about 2 to 2.5 μg/kg/min. Therefore, it seems safe to conclude that extreme asphyxia is a potent stimulant to the adrenal medulla. But, at the same time, it should be emphasized that an accelerated catechol secretion appears to be a rather late event in the asphyxial defensive response.

The relative roles of the direct sympathetic innervation and the adrenal medullary hormones in the responses of some smooth muscle effectors to asphyxia

Method

Experiments were performed on thirty-eight cats anaesthetized as previously described (p. 20). The smooth muscle effectors studied were the muscular blood vessels, the vessels of the skin, the renal vessels, the spleen and the nictitating membrane. The iris was omitted because of its dual innervation. The responses of these effectors were recorded by the same methods that are described in detail under the separate headings in chapter III.

In the experiments on the various vascular regions, the interfering effects on blood flow caused by blood-pressure changes accompanying asphyxia were eliminated, as far as possible, by continuously adjusting a screw clamp applied to the abdominal aorta proximal to the vascular region studied. The 'perfusing' arterial blood

pressure, thus kept approximately constant at about 120 mm Hg, was recorded from a femoral artery or from the caudal extension of the abdominal aorta. In many experiments a blood-pressure fall at the end of the asphyxial period could not be avoided. In these experiments a corresponding reduction of the 'perfusing' pressure was induced by partial occlusion of the abdominal aorta and its interference with the blood flow could thus be evaluated.

Short periods of asphyxia were elicited by clamping the tracheal cannula. At the beginning of each experiment both the corresponding sympathetic innervation of the region investigated as well as the adrenal glands were intact. The effector responses therefore represented the sum of the neurogenic and the humoral sympathetic influences, centrally induced by the stressing of asphyxia. These responses were quantitatively recorded. In the course of the experiment one of the two components was eliminated and asphyxia was again induced. In some experiments the discharge from the adrenal glands was first eliminated, in others the sympathetic innervation of the region investigated. In the course of the experiment the other component was eliminated too. Thus the relative effects caused by the direct sympathetic innervation and the secretion from the adrenal medullae could be quantitatively evaluated in the responses of the various effectors to asphyxia.

Results

A. *The blood vessels of skeletal muscles*

Seven cats were used in this series. A typical experiment is illustrated by fig. 29. About 45 seconds after the asphyxia was started there was a progressive decrease of blood flow indicating an intense vasoconstriction of the muscular blood vessels. At the height of the asphyxia the peripheral resistance was increased some five times. These effects could be regularly reproduced. The sympathetic innervation to the hind limb was then cut, but the adrenals were left intact, after which a new period of asphyxia was induced. The vasoconstrictor response was now *totally abolished* and, as a matter of fact, a moderate increase of the blood flow was observed. This vasodilatation might be due to an accumulation of metabolites in the skeletal muscles and the blood. It might also, at least in

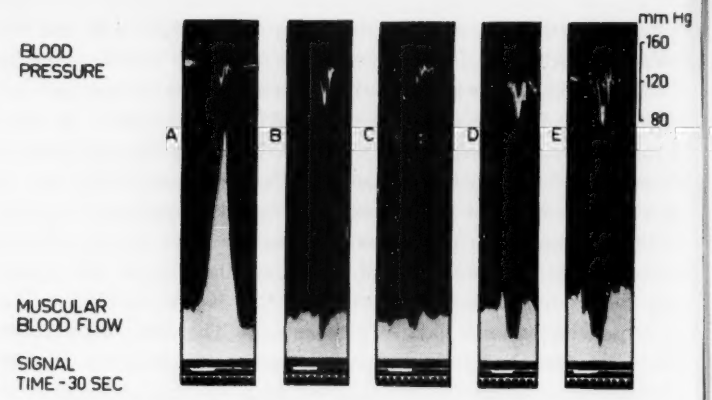

Fig. 29. Adjustments of muscular blood flow during asphyxia. *A* — Asphyxia (1 min, 45 sec) — sympathetic innervation and adrenals intact, *B* — Asphyxia (1 min, 45 sec) — sympathetic innervation cut, *C* — Asphyxia (1 min, 45 sec) — adrenals extirpated, *D* — Asphyxia (1 min, 30 sec) — stim. left splanchnic nerves (10/sec), *E* — Asphyxia (1 min, 45 sec) — injection of catechols (2 μg/kg/min). See text.

part, be attributed to a reflex discharge from the adrenals. There is evidence that *both* factors were responsible for the dilatation as the increase of blood flow was less marked, though still present, when the secretory nerves to the left adrenal were cut and the right adrenal gland was tied off (*C*). At *D* in the same figure a new period of asphyxia was elicited and now the distal ends of the left splanchnic nerves were concomitantly stimulated at 10 impulses per second. The increase of blood flow then became more marked. A similar increase of blood flow was also obtained if the eliminated reflex secretion of the medullary hormones was substituted by an injection of l-adrenaline (30 per cent) and l-noradrenaline (70 per cent) at a total dosage of 2 μg/kg/min during one and a half minute (*E*). This experiment illustrates the dilator capacity of the adrenal medullary secretion even when the noradrenaline percentage is high. However, the intensified discharge from the adrenal medullae was a rather late event and was normally preceded by a pronounced vasoconstriction caused by the sympathetic constrictor nerve fibres. This strong vasoconstriction remained throughout the whole period of prolonged asphyxia. Therefore, the progressive decline of arterial

blood pressure which occurred ultimately in extreme asphyxia could not be attributed to any failure of the vasomotor centre. Furthermore, the neurogenic vasoconstriction was not significantly reduced by the dilator action of the medullary output. The response of muscular blood vessels to asphyxia was quite dominated by a pronounced vasoconstriction effected entirely by sympathetic constrictor nerve fibres to these vessels.

B. *The blood vessels of the skin*

This series included nine cats. In most of these experiments the cutaneous blood flow was recorded separately from both paws of the hind limbs. The sympathetic innervation to the left limb was cut already from the start of the experiment while that to the right limb was left intact. These procedures allowed a concomitant study of the combined effects of the vasoconstrictor nerves and the medullary hormones on the one limb and the hormones only on the other. In other respects the experimental conditions were identical for the two vascular regions.

The profound difference as to the effects of asphyxia is illustrated by fig. 30. The first part of this figure demonstrates that a lowering of the 'perfusing' pressure reduces blood flow to a proportionally larger extent than the decline of pressure, but it affects the two limbs to about the same degree. Part two of this record shows the impressive vasoconstriction caused by the sympathetic constrictor nerves that accompanied the asphyxia. About one and a half minute after the closure of the tracheal cannula the peripheral resistance of the normally innervated cutaneous vascular had increased about fifteen times. The cutaneous blood vessels in the sympathetically decentralized left limb, however, showed only a slight but relatively prolonged vasoconstriction occurring just when the asphyxia was interrupted. This vasoconstriction was probably caused by the adrenal medullary discharge as it disappeared when the adrenal glands were extirpated. The prominent vasoconstriction observed in the sympathetically intact limb, on the other hand, was not to any measureable degree reduced when the adrenal medullary secretion was eliminated. The prominent reduction of blood flow in the paw, which had its vasomotor nerves intact, disappeared when these fibres were cut.

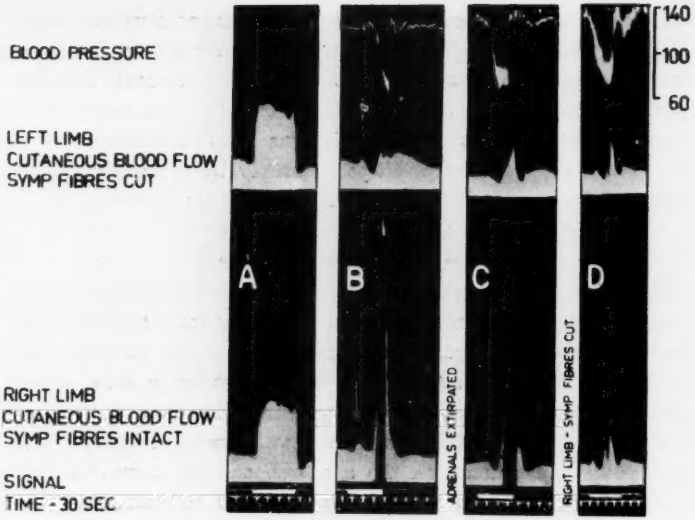

Fig. 30. Adjustments of cutaneous blood flow during asphyxia. See also text on figure and running text. *A* — 'Perfusing' arterial blood pressure lowered by partial occlusion of abdominal aorta, *B* — Asphyxia (1 min, 45 sec), *C* — Asphyxia (1 min, 45 sec) — adrenals extirpated, *D* — Asphyxia (1 min, 45 sec) — sympathetic innervation cut right limb.

Thus, the adjustments of the skin vessels, effected by the sympathetic nervous system in response to asphyxia, were quite dominated by the effects of the direct vasoconstrictor innervation, whereas the effects of the adrenal medullary secretion were relatively insignificant.

C. *The blood vessels of the kidney*

Experiments were performed on six cats. The renal vasoconstriction induced by asphyxia was surprisingly intense in view of the previous difficulties to elicit a vasoconstriction of the renal vessels by stimulation of the sympathetic renal nerves. As mentioned before, it is, however, very difficult to be sure that *all* constrictor nerve fibres to the renal vessels were isolated and excited in those experiments.

The strong renal vasoconstriction that could be reflexly induced by asphyxia is illustrated by fig. 31. About two minutes after the

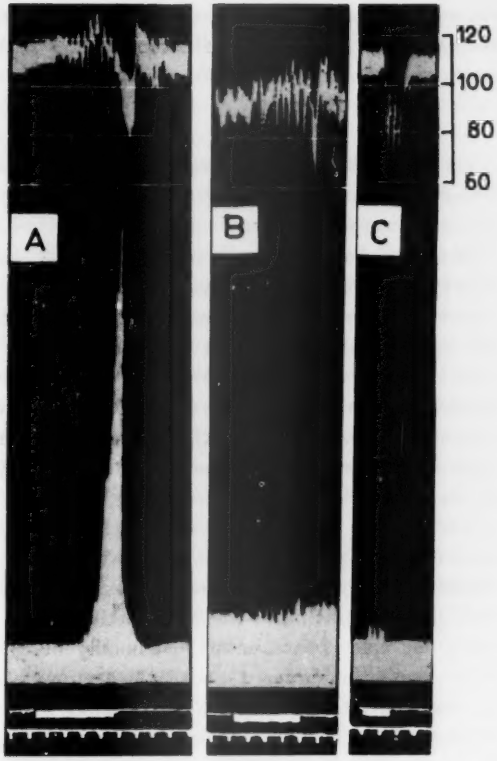

Fig. 31. Adjustments of renal blood flow during asphyxia. *A* — Asphyxia (2 min, 15 sec) — sympathetic innervation and adrenals intact, *B* — Asphyxia (1 min, 50 sec) — sympathetic innervation cut, *C* — 'Perfusing' blood pressure lowered by partial occlusion of abdominal aorta.

closure of the tracheal cannula the peripheral resistance within the renal vascular area was increased some ten times as compared with initial level. The intense renal vasoconstriction accompanying asphyxia indicates a marked participation of this vascular region in the asphyxial 'emergency reaction'. However, if the sympathetic renal nerves were transected the renal vasoconstrictor response to asphyxia was absent or very slight in spite of the asphyxial excitation of the adrenal medullae. See *B* in the same fig. There was thus

not much evidence that the role of the adrenal medullary hormones in the renal vasoconstriction accompanying asphyxia was of any major importance.

D. *The spleen*

The contractions of the spleen, effected by the sympathetic nervous system in response to asphyxia, were recorded in nine cats using the same method as described on page 69.

In the previous series concerning the blood flow through skeletal muscles, the kidney, and to some extent also the skin, there mostly occurred only a moderate increase of blood flow when the corresponding sympathetic innervation was cut. During 'basal' conditions therefore, and as long as the general condition of the animals was satisfactory (to judge from e. g. the blood-pressure rise upon occlusion of the carotid arteries), the 'neurogenic' tone on these effectors appeared to be moderate, corresponding to a low discharge rate of the constrictor nerve fibres.

The spleen, on the other hand, generally expanded markedly subsequent to sympathectomy. This divergence as to the different smooth muscle effectors does not necessarily imply that the discharge rate of the splenic nerve fibres is especially high. It is possible that the tonic splenic contraction, commonly observed, was due to a reflex excitation induced by the laparotomy. But it should also be considered that the spleen is extremely sensitive to the activity of the splenic nerves, and it was shown previously in this study that, already at stimulation rates below one impulse per second, the spleen could be made to contract markedly. The splenic dilatation which commonly followed the transection of the splenic nerve fibres indicates that the size of this organ during basal conditions is primarily dependent on the direct sympathetic innervation.

A comparison of the effects produced on the spleen by the splenic nerve fibres and the medullary hormones separately during strong asphyxia will, however, be somewhat complicated by the fact that the initial size of the spleen was widely different in the two instances.

If the splenic nerves were intact but the adrenals tied off the spleen remained moderately contracted already before the asphyxia was initiated. Any further contraction of the spleen, caused by an

increased discharge rate in the splenic nerve fibres, will thus occur on the upper and more flat part of the 'frequency-response' curve (see fig. 20). Therefore, a marked increase of discharge in the splenic nerve fibres might well provoke only a moderate additional reduction of the spleen size.

But if, on the other hand, the splenic nerves were transected and the adrenals left intact the neurogenic basal tone was abolished and the spleen expanded. The initial size of the spleen before the asphyxia was started was now considerably larger, and the contraction provoked by a release of catechols during asphyxia occurred on the lower and more steep part of the 'dose-response' curve (see fig. 20). This means that a moderate release of catechols from the adrenals will induce a comparatively extensive response.

It follows that it is not quite correct to base a judgement as to the relative roles of the splenic nerves and the medullary hormones during strong asphyxia on the extent of the responses elicited by these two components separately.

In order to get a common reference point for a comparison of the effects of the splenic nerves and the medullary hormones on spleen size during asphyxia, the upper limit of splenic contraction was determined at the end of each experiment. This upper limit was achieved by supramaximal electrical stimulation of the peripheral end of the splenic nerve bundle at a rather high rate (20 impulses per second). A comparison between the splenic surface area that prevailed when the sympathetic nerve fibres to the spleen had been transected, and the surface area that could be determined when the spleen contracted maximally in response to stimulation of the splenic nerves, gave information about the magnitude of the maximal splenic contraction that was possible to provoke.

In five experiments, where the sympathetic nerve fibres to the spleen remained intact but the adrenals were tied off in the course of the experiment, there was a pronounced shrinkage of the spleen that started about 45 seconds after the closure of the tracheal cannula. (It is necessary to be very cautious when the adrenals are tied off because any damage, inflicted on the splanchnic nerves or other sympathetic structures in the vicinity of the adrenals, might interfere with the sympathetic innervation to the spleen.) These splenic contractions were found to be practically maximal when

compared with those obtained in response to splenic nerve stimulation at the termination of the experiments. Further, the extent of the neurogenic contractions to asphyxia was unchanged whether the adrenal glands were left intact or not.

In the remaining four experiments of this group the splenic nerves were cut from the start, but the adrenals were left intact. Under such circumstances a moderate contraction of the spleen occurred in response to asphyxia; an effect which must be attributed to the discharge from the adrenal medullae. To judge from the splenic response, the catechol secretion induced by asphyxia was still a rather late event. A splenic contraction was generally not established until artificial respiration was commenced. Furthermore, the medullary hormones alone did never effect such a forceful contraction as did the direct sympathetic innervation. The magnitude of the splenic contractions induced by the adrenal catechols averaged some 50 to 65 per cent of the maximal response that followed upon stimulation of the splenic nerves.

The importance of the adrenal medullary hormones in the sympathetic control of splenic volume is rather difficult to evaluate. There was, however, no evidence that the adrenal medullae contributed to the tonic contraction of the spleen which was commonly observed during basal conditions. As to the splenic response to asphyxia in animals with the splenic nerves left intact, the neurogenic contraction occurred with a much shorter latency than the humoral effects of the medullary hormones and rapidly reached a maximum. Thus, in the intact organism the medullary catechols, reflexly released by asphyxia, will affect a tissue that is already practically maximally contracted by the activity of the direct sympathetic innervation, and consequently, their effects will be rather superfluous. It is obvious, however, that the medullary secretion of catechols, during extreme asphyxia will be high enough to affect a rather marked contraction of the *sympathetically decentralized* spleen.

E. *The nictitating membrane*

This series included seven cats. The contractions of the membrane were recorded as previously mentioned (p. 21). When compared with the extensive contractions recorded when the cervical sympathe-

tic trunk was stimulated at very low frequencies (see p. 79) the moderate responses of the nictitating membrane to asphyxia were remarkable. Quite commonly the contractions in response to frequencies below one impulse per second were more prominent than those observed in response to even extreme asphyxia. This observation appears to indicate that the nictitating membrane is not, to any larger extent, influenced by the asphyxial type of 'emergency'. However, in spite of this the dominance of the direct sympathetic innervation was still evident.

If both the corresponding sympathetic innervation and the adrenals were left intact there was a moderate contraction of the membrane, after a latency of about one and a half minute, when asphyxia was induced. These contractions were not reduced to any appreciable extent after the adrenal medullary secretion was eliminated. But if, on the other hand, the cervical sympathetic trunk was cut but the adrenals left intact the latency was prolonged and not until two to three minutes of asphyxia had passed did the acutely decentralized nictitating membrane show a small contraction. In three animals the membrane failed to contract altogether in response to the asphyxial medullary secretion of catechols.

Comments

In the present series of experiments the catechol secretion, that was observed during asphyxia, comprised some 20 to 40 per cent of the previously calculated maximal capacity of the adrenal medullae. It was found that in order to achieve this relatively large secretion the asphyxia had to be prolonged until it implied a serious threat to the organism.

As to the relative proportions of adrenaline and noradrenaline in the asphyxial medullary output the indicator used in this study yielded no information. This matter has been investigated recently by Euler and Folkow (1953) and by Redgate and Gellhorn (1953). The former authors found that in the cat the catechol secretion induced by asphyxia averaged approximately 1 μg/kg/min and 78 per cent of this amount was identified as noradrenaline. This was approximately the same proportion as determined on the 'resting' secretion.

The activation of the adrenal medulla that follows upon asphyxia is probably the most intense that can be reflexly provoked (see e. g. the papers by CANNON and HOSKINS 1911, CANNON and CARRASCO-FORMIGUERA 1922, HARTMAN, MCCORDOCK and LODER 1923, CRILE, ROWLAND and WALLACE 1923, KODAMA 1924, HOUSSAY and MOLINELLI 1926, SATO, INABA and TAKAHASHI 1932, RAPELA and HOUSSAY 1952 and EULER and FOLKOW 1953). Asphyxia, therefore, probably brings about a rather generalized excitation of the cell complexes of the adrenal medulla. But in spite of this, the importance of the medullary catechols in the asphyxial defensive reaction is, from a quantitative point of view, insignificant, when compared with the nervous motor control of the various smooth muscle effectors included in this study. On the other hand, it is obvious that a reflex release of adrenaline might effect important metabolic adjustments, especially in the field of carbohydrate metabolism, for reasons as was previously discussed in this study (p. 85). But in the cat noradrenaline constitutes the major fraction of the catechol secretion during asphyxia. The role of this noradrenaline discharge is more difficult to evaluate.

There are some important facts about the asphyxial release of catechols that should be commented upon. First, the excitation of the adrenal medulla is a rather late event in the asphyxial defensive response. The reflexly released catechols did not affect the different smooth muscle groups studied (whether normal or sensitized) until one and a half up to two minutes of asphyxia had passed. This fact might, of course, in part depend on the 'circulation time' from the adrenals to the effectors and this latency might also be prolonged by the circulatory failure quite regularly observed at the height of asphyxia. But the fact remains that the medullary secretion did not reach concentrations that were definitely effective, unless the asphyxia was extreme. The neurogenic adjustments always preceded and quite overshadowed those of the slower acting blood-borne catechols.

Second, the importance of the asphyxial medullary secretion cannot be decided only from the responses of sympathetically decentralized tissues. It has to be evaluated from its quantitative contribution in the sympathetic 'motor' control of various effectors and in its normal competition with the direct sympathetic innerva-

tion. If this point of view is applied, the relative insufficiency of the medullary hormones is apparent. The outstanding feature in this competition was the profound dominance of the neurogenic component in the responses.

In the skeletal muscles, where the direct innervation and the adrenal medullary discharge opposed each others, the predominance of the direct sympathetic innervation was still very marked.

The relative ineffectiveness of the medullary discharge on many effectors may partly be related to the high percentage of noradrenaline in the asphyxial secretion. The motor activities of this compound seemed to be definitely less pronounced than those of adrenaline (see Chapter III). But it should also be remembered that the constrictor actions of the medullary catechols might, to a large extent, be masked by the opposing effects of metabolites which accumulate during asphyxia.

It has been claimed by MALMÉJAC and CHARDON (1946, 1947) that the central structures that govern the adrenal medullae should be more resistant towards anoxia than were the vasomotor centre. According to these workers, an increased rate of catechol secretion could be demonstrated during prolonged asphyxia, while, at the same time, the arterial blood pressure was steadily declining. This blood-pressure fall was attributed to a failure of the vasomotor centre. Let it be said again, that the blood-pressure record is definitely not a safe indicator on vasomotor nerve activities. It could be clearly established in the present study, adopting methods that allow quantitative continuous measurements of peripheral resistance of various vascular regions, that a fall of arterial blood pressure during asphyxia quite regularly coincided with a prominent *increase* of the vasomotor nerve discharge. Thus, if the peripheral resistance were the sole factor responsible for the arterial blood pressure, the prominent neurogenic vasoconstrictions, which were observed during asphyxia, would have raised the blood pressure up to very high levels. Therefore, the progressive decline of blood pressure at the terminal phase of asphyxia cannot possibly be a consequence of any failure of the vasomotor centre. It is, with all probability, due to a cardiac failure induced by anoxia.

In the present study the sympathetic responses of various smooth muscle effectors to asphyxia could be quantitatively and conti-

nuously recorded, and the rather clear cut experimental circumstances allowed an evaluation of the relative importance of the neurogenic influences, on the one hand, and the humoral effects of the medullary hormones on the other. The predominance of the direct sympathetic innervation in the asphyxial defensive reaction was obvious. This view is also, more or less directly, supported by previous investigators. See e. g. the papers by GLEY and QUINQUAUD (1921), ROGOFF and COOMBS (1923), GANTER (1926), HEYMANS and BOUCKAERT (1934) and also the short review by BRÜCKE (1950). Too often, however, the quantitative evaluations of vasoconstrictor responses were based only upon the blood-pressure record which is a poor and often quite an erroneous indicator in these respects.

CHAPTER VI

General comments and conclusions

In the previous chapters most of the experimental data have already been commented upon. These concluding remarks are just intended to give a general summing up of the main results and to stress the general conclusions which they allowed.

The major aim of the present investigation was to carry out a comparative study of the full range of motor control that each of the two components of the 'sympathico-adrenal' system is capable of exerting on different subordinate smooth muscle effector groups. Besides, some information might be gained concerning their relative efficiency by a comparison of the extent of the responses that they will bring about on different effectors when activated separately at similar rates.

I have been primarily interested in the sympathetic control of the 'small vessels'. Because of various reasons, previously discussed in this study, the arterial blood-pressure record *alone* was considered a definitely unsatisfactory indicator as to what might be going on within the circulatory system in this very respect. Closer information about sympathetically induced motor adjustments of the peripheral circulation can be achieved only by a quantitative analysis of the actual changes of circulatory resistance in different areas.

In order to obtain experimental conditions which allow a mutual quantitative comparison between the responses to the neural and the hormonal component of the 'sympathico-adrenal system' certain precautions became necessary. In the first place, both had to be stimulated separately at similar rates within the physiological discharge range of the sympathetic nervous system. Furthermore, the hydrostatic interference of a fluctuating arterial blood pressure had to be eliminated. The importance of this procedure will be evident from a consideration of e. g. the study by GREEN, LEWIS,

Nickerson and Heller (1944) and the concept of the 'critical closing pressure' as introduced by Burton (see e. g. Burton 1951). Therefore, in the present investigation all changes of the rate of blood flow in different vascular regions were recorded at an arterial 'perfusing' blood pressure that was kept constant at about 120 mm Hg. Under such circumstances, all changes in regional circulatory resistance will be approximately inversely proportional to the rate of local blood flow. Finally, the experiments had to be undertaken on isolated vascular regions acutely decentralized from the sympathetic nervous system.

These steps enabled that the effects on a certain vascular region — whether elicited by stimulation of the corresponding direct sympathetic innervation or the sympathetic secretory nerves to the adrenals — could all be quantitatively recorded under fairly identical experimental conditions. They could furthermore be mutually compared when expressed in terms of 'relative units' for peripheral resistance.

As a comparison — and in order to see if the quantitative relationships found to apply for the blood vessels also hold as regards other smooth muscle effector groups — the nictitating membrane and the dilator muscle of the iris were included in this study.

The results of this comparative study were given in Chapter III and the main points were the following. In the first place, on all effectors studied the *total range* of motor control effected by the direct sympathetic innervation was found to be much more extensive than that exerted by the adrenal medullae. So for instance, stimulation of the constrictor nerve fibres to a cutaneous vascular region in some animals reduced blood flow almost to a standstill which implied roughly a hundredfold increase of the peripheral resistance. A maximal adrenal medullary discharge, on the other hand, never increased the peripheral resistance of the same vascular region more than some ten times. Likewise, when the sympathetic nerve fibres to any effector and the adrenal secretory nerves were exited separately at the *same rate* the neurogenic predominance was always very marked. In most instances, drastic motor adjustments occurred in response to low frequency stimulation of the sympathetic nerve fibres, whereas the effects related to the adrenal medullary catechols appeared moderate or small. Regarding e. g. the cutaneous blood vessels the effects obtained by the direct innervation were roughly

10 to 20 times greater than those elicited by the adrenal medullary output. If both the neural and hormonal component were stimulated *simultaneously* and at the *same rate* (e. g. 2 to 4 impulses per second) the hormonal component did not, to any significant degree, reinforce the effects of the neural component. Only by stimulating the adrenal secretory nerves at rates several times higher than those applied to the direct sympathetic nerve fibres was it possible to provoke a clear cut reinforcement which could be related to the medullary catechols.

However, there seems to be little experimental evidence to support the view that in various states of a reflex activation of the sympathetic nervous system the adrenal medullae alone should be heavily excited, while at the same time, the neurogenic component should be little influenced. Especially at intense activation — as seen during a multitude of stressful conditions — the sympathetic nervous system is brought into action as a whole, influencing practically all subordinate tissues and probably than at a rather uniform rate of discharge.

The suggestion that 'sympathin' by an 'overflow' mechanism will reach the blood in amounts high enough to significantly reinforce the effects of the direct sympathetic innervation and the medullary catechols (see e. g. ROSENBLUETH 1950) was not supported by the experiments reported in Chapter IV. The idea of an 'overflow' mechanism goes back to the intricate hypothesis suggested by CANNON and ROSENBLUETH (see e. g. CANNON and ROSENBLUETH 1937) about the 'key cell' arrangement of the peripheral autonomic innervation. This hypothesis has not withstood the experimental evidence and criticism brought forward especially by HILLARP (1946). Contrary to the 'key cell' theory there is now a rather solid ground for the assumption that every cell, subordinate to the sympathetic nervous system, makes a close contact with sympathetic nerve fibres, and, as a matter of fact, an overlap appears to exist, so that axon ramifications from several neurons converge upon each effector cell. See e. g. the papers by ECCLES and MAGLADERY (1937 a, b) and HILLARP (1946). There is further good evidence of the existence of an enzymatic inactivation of the adrenergic transmitter substances locally at the site of their release. See e. g. THOMPSON and TICKNER (1951), and the short reviews by BURN (1952) and by BLASCHKO (1953).

In the present study there was a total failure to provoke 'remote effects' due to 'sympathin' on all acutely sympathetically decentralized effector groups — and, in most instances, also on the highly sensitized denervated nictitating membrane — even when a major tissue area was intensely excited by high frequency stimulation of the corresponding sympathetic nerve fibres. In addition, the observation was made that catechols when infused into the lower abdominal aorta did not reach the venous blood in effective concentrations until fairly large amounts were given. These findings support the view of a peripheral inactivation mechanism which then largely eliminates the possibility of a 'sympathin' overflow.

Thus — regarding the sympathetic motor control of various smooth muscle effectors — a closer analysis concerning the relative potency of the three possible ways of sympathetic ergone supply revealed that the direct sympathetic innervation was by far the strongest competitor. The importance of the adrenal medullae was fairly limited in this respect and the 'overflow' mechanism might, from a quantitative point of view, be completely ignored.

To see if the abovementioned quantitative relationships are valid also in a true reflex excitation of the sympathetic nervous system, the motor adjustments of various effector groups in response to asphyxia were investigated (Chapter V). This type of reflex excitation was chosen because it is known to induce an intense activation of the adrenal medulla, thus putting this component in a relatively favourable competitive position. The predominance of the direct sympathetic innervation was still very pronounced. (The functional differentiation of the adrenal medulla in an adrenaline- and a noradrenaline-producing fraction — which under many other circumstances seem to be separately activated — probably implies that in many types of reflex adjustments the competitive position of the adrenal medulla is less favourable as then only a fraction of the gland cells are activated.)

On the basis of the present findings it seems obvious that the motor control of smooth muscle effectors subordinate to the sympathetic nervous system is quite dominated by their corresponding sympathetic innervation whereas the contributary influences of the medullary catechols appear to be of little quantitative importance. Much of the previous literature support this view. See e.g. the

papers by GLEY and QUINQUAUD (1917/18 and 1921), ROGOFF and COOMBS (1923), GANTER (1926), HEYMANS (1929), HEYMANS and BOUCKAERT (1934). CANNON and his group (see e.g. CANNON and ROSENBLUETH 1937), on the other hand, seem to put stress on the importance of the adrenal medulla and also on the effects of circulating 'sympathin'. However, either the usage of the arterial blood-pressure record as the only indicator as to the extent of circulatory changes, or the lack of a standard of comparison regarding the effects of the direct sympathetic innervation *versus* the adrenal medulla, makes most of the previous studies far from conclusive regarding the actual quantitative importance of the adrenal glands.

When discussing the probable physiological significance of the adrenal medullary hormones it should be kept in mind that the adrenal medulla holds a unique position in the sympathetic nervous system. Whereas the adrenergic transmitter substances, most probably, exert a fairly localized action — mainly restricted to the effector cells innervated —, the adrenal medullary hormones, on the other hand, will be diffusely distributed and are thus capable of affecting practically every cell in the organism. Consequently, the prime importance of the medullary hormones might be looked for in tissues which *lack* a direct sympathetic innervation and where the competition with the neurogenic component is therefore not met with.

This fact focuses the attention on the 'metabolic' actions since long ascribed to the medullary hormones. The 'metabolic' part of the sympathetic control has not been studied in the present investigation. The available literature on these matters, however, brings forward certain characteristics. In the first place, these 'metabolic' effects — as for instance the mobilization of glucose from the liver cells and the break-down of glycogen into lactic acid in the skeletal muscles — occur in tissues which, most probably, are *not* under a direct nervous control of the sympathetic nervous system. They also appear to be ultimately dependent upon the integrity of the adrenal glands and are more or less totally abolished when these glands are excluded. So for instance it was noticed already by CANNON, STOHL and WRIGHT (1911) that the glucosuria that followed upon emotional excitation of cats disappeared when the adrenals were extirpated. Similar observations were reported after exposure to asphyxia (KAHN 1912, STARKENSTEIN 1912 and OLMSTED 1925).

JAEGHER and VAN BOGAERT (1935) noticed that, whereas the pressor response to electrical stimulation of the hypothalamic region was not modified by extirpation of the adrenals, the impressive rise in the blood-sugar level disappeared. A closer analysis of the neural and hormonal factors in emotional hyperglucaemia was published by BRITTON (1928). He reported that severance of the splanchnic nerve fibres to the liver only slightly modified the hyperglucaemic response to emotional excitation in cats. In contrast, 'profound disability of the glycogenolytic mechanism' was observed in animals deprived of the adrenal medullae but with the liver nerves intact.

Another characteristic feature of the 'metabolic' effects is the fact that small amounts of adrenaline — well within the lower physiological discharge range of the adrenal medullae — might bring about important effects. See e. g. the papers by CORI, CORI and BUCHWALD (1930), CORI and BUCHWALD (1930), GRIFFITH, LOCKWOOD and EMERY (1939), LUNDHOLM (1949 b), BEARN, BILLING and SHERLOCK (1951), PEKKARINEN and HORTLING (1951) and the review by GRIFFITH (1951).

Still another important fact is that metabolic effects in general appear to be primarily mediated by adrenaline but rather poorly effected by noradrenaline (see e. g. SCHÜMANN 1949, BEARN et al. 1951 and BLOOM and RUSSELL 1952). So for instance SCHÜMANN reported that the capacity of l-adrenaline to raise the blood-sugar level was about 20 times that of dl-noradrenaline.

These various types of experimental evidence indicate that the metabolic effects of the sympathetic control are primarily mediated by way of the blood-borne adrenaline hormone. From a teleological point of view such a functional differentiation between the two efferent links of the 'sympatho-adrenal' system is rather suggestive. The fast adjustments in the sympathetic motor control — as seen for instance in variations of posture of the individual — might well be explained by rapid changes in impulse discharge in the thoraco-lumbar sympathetic outflow. The slower and more protracted fluctuations of the metabolic processes might correspond to the slower acting hormonal influences. These metabolic effects might further be marked at a fairly moderate adrenal medullary secretion which probably does not significantly interfere with the rapidly occurring nervous adjustments of the smooth muscle effectors.

As mentioned, the only smooth muscle effector group, on which adrenaline and the adrenal medullary output *did* show quantitatively very important effects, was the blood vessels of skeletal muscles. This dilatation of muscular blood vessels was entirely related to adrenaline whereas noradrenaline proved ineffective. It was further pronounced already at a minute concentration of adrenaline. At the same time adrenaline at higher concentrations constricted the muscular blood vessels, and in this respect it was not less potent than noradrenaline. The present observation that 'close arterial' administration of minute amounts of adrenaline to a skeletal muscle region might induce a sustained vasodilatation suggests that the mechanism of the dilator action of adrenaline must be looked for primarily in the skeletal muscle region itself. Thus, the dilator action of adrenaline on muscular blood vessels and the lactacidaemic effects of adrenaline appear to have certain characteristics in common. Besides the fact that both occur in the skeletal muscles, both seem bo be pronounced already at a very low concentrations of adrenaline whereas they are poorly effected by noradrenaline. (A dilator action of noradrenaline, though weak, might of course exist, but is then masked by the concomitant constrictor action of this compund.) Therefore, the hypothesis put forward by LUNDHOLM (1951) on this dilator action of adrenaline was considered the most plausible one. He suggested that the dilator action of adrenaline on muscular blood vessels is connected to its glycogenolytic power and ultimately related to a release of lactic acid in the surrounding skeletal muscle cells. In that case this dilator action of adrenaline is more to be considered a consequence of its 'metabolic' actions rather than representing a true 'motor adjustment' due to adrenaline.

The denotion the 'sympathico-adrenal system', used as a synonym for the two efferent links of the sympathetic nervous system, implies that the neural and the hormonal fraction of this system are tied together into a unit, and it has been commonly understood that both components fairly indiscriminately influence subordinate tissues. On the basis of important divergencies in the structural and functional organization of these two components, and, on the basis of a quantitative analysis, presented in this study, as to their range and relative potency in the motor control of various smooth muscle effectors, it is suggested that there exists a clear-cut functional

differentiation of the 'sympathico-adrenal system'. Thus, the motor control of subordinate smooth muscle effectors, such as the blood vessels, appears to be quite dominated by the neural component. There are good reasons to assume that a corresponding predominance in favour of the adrenal medullary hormones — and then especially adrenaline — applies as to the sympathetic control of various metabolic processes.

Summary

The present study makes up a quantitative analysis as to the range of control that each of the components of the 'sympathico-adrenal system' is capable of exerting on different vascular regions and other smooth muscle effectors. The outstanding predominance of the neural component in the motor control of subordinate effectors was stressed. From a quantitative point of view the effects of the adrenal medullary hormones are insignificant in this respect. It rather seems as if the prime field of action of the medullary secretion is a sympathetic hormonal control of various metabolic processes in tissues — such as skeletal muscle and liver cells — which lack a direct sympathetic innervation. There seems to be no evidence that circulating 'sympathin' is of any importance in either of these respects. A clear-cut functional differentiation between the neural and the hormonal fraction of the 'sympathico-adrenal system' is therefore suggested.

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